

REMARKS

The Office Action has been carefully studied. No claim is allowed. Claims 1, 7-10, 12-16, 25, 28, 38, 39, 41, 42, 46, 48-50, 52, and 56-60 presently appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

The face-to-face interview among Dr. Wu, one of the present inventors, and the undersigned, representing applicants, and Examiners Dr. Kaushal and Dr. Fredman on August 3, 2004, is hereby gratefully acknowledged. Applicants' representatives wish to thank the examiners for the courtesies extended during this interview.

No specific prior art was discussed at the interview as there were no prior art issues raised in the Office Action. All the claims however were discussed, and applicants' representatives agreed to cancel the claims directed to a DNA molecule or convert them to method claims. Applicants' representatives further presented evidence on the interchangeability of uromodulin promoters among mammalian species and also proposed to limit the claims to "mammalian uromodulin promoters", to delete the recitation of "ancestors" from the claims to transgenic non-human mammals, and to incorporate the recitation of expression of the heterologous polypeptide "to the ascending limb of Henle's loop and to the early distal tubules of the kidneys" in method claim 38 in order to overcome the enablement rejection. Agreement was reached that the enablement rejection would likely fall.

The written description rejection was also discussed at the interview, with applicants' representatives arguing that there is a

sufficient representative number of uromodulin promoter sequences identified in the instant specification and in the prior art to satisfy the written description requirements. The only agreement reached on this written description issue was that applicants would introduce new claims directed to specific mammalian uromodulin promoters and specific transgenic non-human mammals and the examiners would carefully consider this issue in view of applicants' arguments in the instant amendment. The argument presented at the interview as it relates to the written description rejection is incorporated below.

Claims 41 and 55 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement (new matter issues).

The recitation of "pig... uromodulin promoter" is now deleted from claim 41, thereby obviating this part of the rejection. Claim 41 is now amended to recite "human... uromodulin promoter" in place of pig uromodulin promoter as supported on page 19, lines 15-27 of the specification.

The feature of "mammalian uromodulin promoter directs expression of said heterologous polypeptide *in vivo* to the ascending limb of Henle's loop and the early distal tubules of the kidneys" as now recited in claims 38, 57 and 59 (claim 55 is now cancelled) is supported on page 14, lines 23-26 and page 16, lines 21-27.

Reconsideration and withdrawal of this rejection are therefore respectfully requested.

Claim 8 has been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for the recitation of "predicted β -

turns". This rejection appears to no longer be an issue as the examiners have accepted applicants' argument that predicting the location of β -turns using common and publicly available algorithm, such as the Chou-Fasman algorithms, among others, is well recognized by those of skill in the art and therefore is not indefinite even if the specific algorithm is not mentioned by name in the specification.

Claims 1, 7-10, 12-16, 29, 31-37, 47 and 55 have been rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. Claims 25, 28, 38-42, 44, 46, and 48-54 have also been separately rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. Both of these rejections are obviated by the amendments to the claims as discussed at the face-to-face interview on August 3, 2004, the evidence on interchangeability of mammalian promoters as presented below in applicants' arguments rebutting the written description rejection, and the merely routine experimentation that is required by those of skill in the art to obtain transgenic non-human mammals.

Claims 1, 7-10, 12-16, 25, 28-29, 31-42, 44, and 46-55 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is respectfully traversed.

The rejection as it relates to the scope of the transgenic animals and as discussed at the interview no longer appears to be at issue, particularly in view of the species of mammals recited in the claims being well described in the specification and further in view of

the examiner's agreement that enablement with regard to the different species of transgenic non-human mammals would be withdrawn as a result of the amendments to the claims.

The examiner holds that, with regard to uromodulin promoters, the instant specification only teaches the murine, goat and bovine uromodulin promoters but fails to disclose uromodulin promoter sequences and states, "Beside the mouse uromodulin promoter comprising the nucleotide sequences of SEQ ID NO:1, the specification as filed fails to disclose any other uromodulin promoter or fragment thereof (obtained from any amphibian, reptile, bird or all mammal) that directs the expression of a polypeptide *in vivo* in the kidney to produce the polypeptide in the urine."

In response to the examiner's position in this part of the rejection, applicants have now amended the claims to recite for only "mammalian" uromodulin promoters. As disclosed in the present specification at page 19, lines 15-18, the sequence of the mouse and goat uromodulin promoters have now been determined and reported by the applicants in the instant specification and the bovine, rat and human promoter regions have been previously reported in the prior art. On page 18, lines 15-19, a Yu et al. (1994) reference is cited for its disclosure of the bovine and rat (and human) uromodulin promoter regions and is furthermore incorporated by reference. This Yu et al. (1994) reference is the Yu et al., "Bovine and rodent Tamm-Horsfall protein (THP) genes-cloning, structural analysis, and promoter identification", Gene Expression 4:63-75 (1994) reference AM submitted with the Information Disclosure Statement dated July 13, 2000. For

clarification, uromodulin is also known as Tamm-Horsfall protein (THP). Fig. 5 in this Yu et al. (1994) reference shows a sequence alignment of the promoter regions of bovine, rat and human uromodulin. Accordingly, the uromodulin promoters from mouse, goat, cattle, rat, and human are disclosed in the instant specification and provides a representative number of species of the genus of mammalian uromodulin promoters so as to reasonably convey to those of skill in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. This is further supported by the following discussion below.

Zbiskowska et al. (2002), cited by the examiner, discloses that a human uromodulin promoter provides kidney-specific expression in transgenic mice that leads to production of a recombinant biologically active human antitrypsin in the urine. Zbiskowska arbitrarily chose a human uromodulin promoter region that also contains exon 1 and part of exon 2 to drive expression, even though the exon sequences are unnecessary for kidney-specific expression. This can be seen from applicants' own evidence (Zhu et al., 2002 and 2003, copies of which were submitted with the amendment filed July 28, 2003), where sequences from only the 5'-upstream non-coding region were found to be sufficient for directing high level kidney-specific expression.

The Kim et al., Transgenic Res. 12:191-201 (2003) reference, which was also submitted with the amendment of July 28, 2003, demonstrates that the most critical cis elements of the uromodulin gene are located within the 600 bp upstream region (see abstract), providing evidentiary support for applicants' expectation in the specification at

page 18, lines 23-28, that the approximately 600 base pairs of sequence upstream of the uromodulin coding sequence is sufficient for directing kidney specific expression. Moreover, like Zbiskowska's showing of a human uromodulin promoter being capable of kidney specific expression in mice, Kim et al. also establish that a bovine uromodulin promoter can likewise function in mice. These results demonstrate the interchangeability of mammalian uromodulin promoters from one mammalian species to another, which is altogether expected given the disclosure in the specification at page 17, lines 13-15, that uromodulin promoter sequences are likely to be conserved among mammals and the sequence conservation actually observed among the disclosed bovine, rat, human, mouse and goat uromodulin promoter sequences.

To extend the alignment for bovine, rat and human uromodulin promoter sequences in Fig. 5 of Yu et al. (1994), applicants have prepared a comparison/alignment of about 600 base pairs upstream of the uromodulin coding sequence from all five bovine, rat, human, mouse and goat uromodulin promoters. The first alignment attached hereto for the examiner's consideration is entitled "Highly conserved nature of the proximal promoter sequences of rat, mouse, cattle, goat and human uromodulin (Tamm-Horsfall protein)" and was generated using a PileUp program of an online sequence analysis software (SeqWeb). A consensus sequence, shown as the bottom line in the alignment, is readily generated from the alignment of uromodulin promoter sequences from the five mammalian species. The second sequence alignment attached hereto shows the conserved transcription factor binding sites in the proximal promoters of uromodulin.

Also attached hereto are computer printouts of sequence identity calculations (BestFit) between the approximately 600 bp uromodulin promoter sequences presented in the first and second sequence alignments. A table summarizing the sequence identity calculated between each of five uromodulin promoter sequences is provided as a further attachment for the examiner's consideration. This table shows that the level of sequence identity is exceedingly high for promoters, ranging from 73% to 94% over approximately 600 base pairs.

On page 14, lines 17-20 of the instant specification, it is disclosed that uromodulin (Tamm-Horsfall protein) is by far the most abundant urinary protein of human and higher mammals, with an excretion rate of up to 200 mg per day.

In conclusion, when those of skill in the art take the five disclosed mammalian uromodulin promoters altogether into consideration along with (1) the high sequence identity between the five identified uromodulin promoters (sufficient to generate a consensus sequence), (2) the expectation that other mammalian promoters would have similar high sequence identity to the five identified mammalian uromodulin promoters and the consensus mammalian uromodulin promoter, (3) the functional interchangeability of the mammalian promoters among mammalian species, and (4) the ease in readily identifying a uromodulin promoter based not only on its sequence (i.e., sequence identity) but also on its ability to drive the expression of the most abundant (and therefore easily detectable) urinary protein in mammals for secretion into the urine at the ascending limb of Henle's loop and the early distal tubules of the

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kidneys, these same persons of skill in the art would immediately recognize that the inventors, at the time the application was filed, had possession of the claimed invention.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 U.S.C. §112 and define patentable subject matter warranting their allowance. Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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HIGHLY CONSERVED NATURE OF THE PROXIMAL PROMOTER SEQUENCES
OF RAT, MOUSE, CATTLE, GOAT AND HUMAN UROMODULIN (TAMM-
HORSEFALL PROTEIN)

	1		31
RAT	C TAGTCTTGT. CTGACAGAGG TCCAGTTGAG		
MOUSE	C CAGTCTTGT. CTGACAGAGG TCCAGTTGAG		
CATTLE	C TGGTCCAATG ATGTCTGAAT TGCCTTCTGT		
GOAT	C TGGTCCAATG ATGTCTGAAT TATCTGCTGT		
HUMAN	C AGGTCCAGTG ATGTCTGAAC TACCTTCTGG		
CONSENSUS	C TGGTCCAGTG ATGTCTGAA- T-CCTTCTGG		
	32		81
RAT	GGATGTCCAG ATGGTCTTGC AACC.GATAA CTTTCTCAGA GACTCTCTCT		
MOUSE	GAGTGTCCAG ATGGTCTGAT AACCTGATGC CATTCTCAGA GA....CTCT		
CATTLE	CTCTGACCTT CAGGCATTCT CAGCTCCTTT CCTGCTCACA TCGGGACCCC		
GOAT	CTCTGACCTT CAGCCATTCT CAGCTCCTTT CCTGATCACA TTGGGACCCC		
HUMAN	TTCTGACTTT CAGCCATTCT CAGCTCCTCT CTTGCTTG TG TCTGGATTCT		
CONSENSUS	-TCTGACCTT CAGGCATTCT CAGCTCCTTT C-TGCTCA-A T-GGGACTCT		
	82		131
RAT	TTCTGTCTG GACTCTAGTG GGGAGGACTA ATCTGGTGAA GCTGTTCTTC		
MOUSE	TTCTGTCTG GAATCTAGTG AGGAGGACTT ATCTGGTGAA GCTGTCCTTT		
CATTLE	AGGGAAGCTG GTTGAACCCA TGAGGATGGA ACTTGCTTTG GAACTGAGTG		
GOAT	AGGGGAGCTG GCTGAATCTG TGAGGATGGC ATTTGCTTTG GAATTAAGTG		
HUMAN	AAGGCTGATC TCATGAGAAT GGGTGTTTCA GAAGGGTGCC CTCTCCA...		
CONSENSUS	A-GGT-GCTG G--T-AA-TG -GG-G-T--A AT-TGGTG-- G---TCA-TG		
	132		181
RAT	AGATCAGGTG TGTGTTCCAG GCTTCGAAGC AAATGTTTCT GTTATCCTAA		
MOUSE	AGAACAGGAG TGTGTTCCAG TCTTCAAAGC AAACATTCTT TTTATCCTAA		
CATTLE	GCCACAAGTA TACATCCCAG TGGGGACAGT GAGCACCCCT TTTCTCCTGG		
GOAT	GCCACAAGTA CACATCCTGG TGGGGACGAT GAGCACCCCT TTTCTCCTGG		
HUMAN	.AGACAGGTG CACCTCCCAT CTGGGGCAGT GAATA.TCCT TTTGTCTTA		
CONSENSUS	---ACAGGTG TAC-TCCCAG T-GGGACAGT GAACA-TCCT TTT-TCCT-A		
	182		231
RAT	CCCAGGCTGG CTTCAGATAT ..TGTCTTTT TTCCTGCCCC TTTGG..TAT		
MOUSE	CACAGTCTGA CTTCAGATAT ACTGTCTTTT TCCTGGCTCC TTGGGCTTAG		
CATTLE	AGCAGCCTGG CTTCAGATT. .CTGGCCTCT GCTT...T..		
GOAT	AGCAGCCTGG CTTCAGATT. .CTGGCCTCT GCTTGGCT..		
HUMAN	TGCAGCCTGG CTTCAGATA. .CTGGCTTCT GCCTGGCTCC TTG.....A		
CONSENSUS	-GCAGCCTGG CTTCAGATAT -CTGGCTTCT GCCTGGCTCC TTGGG--TA-		
	232		281
RAT	TTCCACCTTG TCCTTGCCCA GGTCCAAGAA AAAGCCCAGA ACCTTGGCAC		
MOUSE	GTCTACCTTG TCCTTGCCCA GGTCCAAGAA AAGGCCAGAA ACCTTGGCAC		
CATTLE	..CCACTTTG TGCTTTTCAA TGACCAAGAA .AAGCCCAGG CACTTGGGAAT		
GOAT	..CCACTTTG TGCTTTTCAA TGACCAAGAA .AATCCCAGG CCCTTGGGAAT		
HUMAN	TCCCACCCTG CCCTTGTCAG TGACCAAGAA GAAGCCCAGC ACCTTGGCAC		
CONSENSUS	TTCCACCTTG TCCTTGTCAA TGACCAAGAA AAAGCCCAG- ACCTTGGCAC		

	282		331
RAT	TGCTTTGCCA	GTTAATGTCT	AACCGAGGAA TGTCTTGCTG CCAAAAGGTG
MOUSE	TGTTTTGCCA	GTTAATGTCT	AACTGAGGAA TGTCTTGCTG CCAAAAGGTG
CATTLE	TTTTTACCCA	GTTAATTTCT	AACTAAAGAA CCTCTCGTTG CCAAAAGATA
GOAT	TGTTTACTCA	GTTAATTTCT	AACTAAAGAA CCTCTTGTTG CCAAAAGGTA
HUMAN	TGCTTTCCCA	GTTAATTTCT	AACTATGGAA TCTCTTGCTG TTAGAAGGTG
CONSENSUS	TGTTTTCCCA	GTTAATTTCT	AACTAAGGAA TCTCTTGCTG CCAAAAGGTG

	332		381
RAT	.CAAACAGAG	ACCTTGATTT	TCCAGGCACA GGTGTGACCC C CAATGTCA
MOUSE	.AAAACAGAG	ACCTTGATTT	TCCAGGCACA GGTGTGACCC C AATGTCA
CATTLE	TAAAACAGAG	CCCTTGTAAC	TCTGGGCACA ACTGTGACCC C AGTGTCA
GOAT	TAAAACAGAG	CCCTTGTAAG	TGTGGGCACA GCTGTGACCC C CATGTCA
HUMAN	CGAAACAGTG	ACCTTGATTT	TCCGGGCACA GGTGTGACCC CCAATGTCA
CONSENSUS	TAAAACAGAG	ACCTTGATTT	TCCGGGCACA GGTGTGACCC C-CAATGTCA

	382		431
RAT	ATCATTTTCT	GTCTCTAACT	ACCAGAGGAA AAACCTAACAA CAACAGCCTC
MOUSE	ATCATTT..T	GTGTCTAACT	CCCAGGGGAA AAACCTAACAA CAACAGACTC
CATTLE	ATCATTTGGG	GTCTCTACCT	ATTAG GGAA AA GAACAA CAACCACCTC
GOAT	ATCATTTGGG	GTCTCTACCT	ATTAG GGAA AA GAACAA CANCCACCTC
HUMAN	ATCATTTGGG	GTCTCTAGCT	ATTA GGAA AA AGAACAA CAACAACCTC
CONSENSUS	ATCATTTGGG	GTCTCTA-CT	ATTAG-GGAA AAAAGAACAA CAACAACCTC

	432		481
RAT	ATGGTTTGGA	AAAGGTGAAC	TCTATGCCAA ATGGGAAGAA AAATTCTGAC
MOUSE	ATGGCTTGGA	AAAGGTGAAT	TCTATGCCAA AAGGGAAGGA AAGTTCT.AC
CATTLE	ACAGCCTAGA	AAAGGAAAAC	ACTGTGTCAA AAGGGAAAAA TATTCC..AC
GOAT	ACAGCCTANA	AAAGGAAAAC	ACTGTGTCAA AAGGGAAAAA TATTCC..AC
HUMAN	ACAGCTTGGA	CAAGGCAAAC	ATTATGCCAG GAGGAAAAAA TATTCC..AC
CONSENSUS	ACAGCTTGGA	AAAGG-AAAC	ACTATGCCAA AAGGGAAAAA TATTCT-AC

	482		531
RAT	CCCCACAGAA	ACAATCTCAA	GAGGCAGAAG CAGAGAATAA TTGGAGG.GA
MOUSE	CCCCACAGAA	ACAATCTCAG	AGGGCAGAAG CAGAGAATAA TCTGAGG.GA
CATTLE	CCCCATTAAA	ATA..ATTAA	GAAACAGAAC CAGAGGATCA TTGGAGGAAA
GOAT	CCCCATTAAA	ATA..ATTAA	GAAACAGAAC CAGAGGATCA TTGGAGGAGA
HUMAN	CCCCAAGAAA	ACAATATCAA	AAAACAGAAC TAGAGACTAA TTGGAGGAGA
CONSENSUS	CCCCA--AAA	ACAATATCAA	GAAACAGAAC CAGAGAATAA TTGGAGGAGA

	532		581
RAT	GAGGGCCAGC	CAAGGGCAGA	CATATATATA TATATATTGA TCACAGGCAC
MOUSE	GAGGGCCAGC	CAAGGGCAGG	CAAGTATATA TTGA TCACAGGCAC
CATTLE	GACTGCCAGT	GGGGGACAGA	TGTATATATA TAGATATGAT AGTCACCTAC
GOAT	GATTGCCAGT	GGGGGACAGA	TGTATATATA TAGATATGAA AGTCACCTAC
HUMAN	GATTGCCAGC	CTGGGGCAAA	TGTGTATATA TAAGTATGAG GCACA.....
CONSENSUS	GA-TGCCAGC	C-GGGGCAGA	TGTATATATA TA-ATATGAA -CACA---AC

	582	591
RAT	TTACTTGTGA	
MOUSE	TTACTTGTGA	
CATTLE	TTGTAAAAGG	
GOAT	TTGTAAAAGG	
HUMAN	TCATCACCAG	
CONSENSUS	TTAT-A--GG	

CONSERVED TRANSCRIPTION FACTOR BINDING SITES IN THE PROMIXMAL PROMOTERS OF UROMODULIN

		1		31
RAT		C	TAGTCTTGT. CTGACAGAGG TCCAGTTGAG	
MOUSE		C	CAGTCTTGT. CTGACAGAGG TCCAGTTGAG	
CATTLE		C	TGGTCCAATG ATGTCTGAAT TGCCTTCTGT	
GOAT		C	TGGTCCAATG ATGTCTGAAT TATCTGCTGT	
HUMAN		C	AGGTCCAGTG ATGTCTGAAC TACCTTCTGG	
CONSENSUS		C	TGGTCCAGTG ATGTCTGAA- T-CCTTCTGG	
	32			81
RAT	GGATGTCCAG	ATGGTCTTGC	AACC.GATAA CTTTCTCAGA GACTCTCTCT	
MOUSE	GAGTGTCCAG	ATGGTCTGAT	AACCTGATGC CATTCTCAGA GA...CTCT	
CATTLE	CTCTGACCTT	CAGGCATTCT	CAGCTCCTTT CCTGCTCACA TCGGGACCCC	
GOAT	CTCTGACCTT	CAGCCATTCT	CAGCTCCTTT CCTGATCACA TTGGGACCCC	
HUMAN	TTCTGACTTT	CAGCCATTCT	CAGCTCCTCT CTTGCTTGTG TCTGGATTCT	
CONSENSUS	-TCTGACCTT	CAGGCATTCT	CAGCTCCTTT C-TGCTCA-A T-GGGACTCT	
	82			131
RAT	TTCCTGTCTG	GACTCTAGTG	GGGAGGACTA ATCTGGTGAA GCTGTTCTTC	
MOUSE	TTCCTGTCTG	GAATCTAGTG	AGGAGGACTT ATCTGGTGAA GCTGTCTCTT	
CATTLE	AGGGAAGCTG	GTTGAACCCA	TGAGGATGGA ACTTGCTTTG GAAGTGAATG	
GOAT	AGGGGAGCTG	GCTGAATCTG	TGAGGATGGC ATTTGCTTTG GAATTAAGTG	
HUMAN	AAGGCTGATC	TCATGAGAAT	GGGTGTTTCA GAAGGGTGCC CTCTCCA...-	
CONSENSUS	A-GGT-GCTG	G--T-AA-TG	-GG-G-T--A AT-TGGTG-- G---TCA-TG	
	132		TCF-1	181
RAT	AGATCAGGTG	TGTGTTCCAG	GCTTCGAAGC AAATGTTTCT GTTATCCTAA	
MOUSE	AGAACAGGAG	TGTGTTCCAG	TCTTCAAAGC AAACATTCCT TTTATCCTAA	
CATTLE	GCCACAAGTA	TACATCCCAG	TGGGGACAGT GAGCACCCCT TTTCTCCTGG	
GOAT	GCCACAAGTA	CACATCCTGG	TGGGGACGAT GAGCACCCCT TTTCTCCTGG	
HUMAN	.AGACAGGTG	CACCTCCCAT	CTGGGGCAGT GAATA.TCCT TTTGTCTTA	
CONSENSUS	---ACAGGTG	TAC-TCCCAG	T-GGGACAGT GAACA-TCCT TTT-TCCT-A	
	182	NF-GMB		231
RAT	CCCAGGCTGG	CTTCAGATAT	..TGTCTTTT TTCCTGCCCC TTTGG..TAT	
MOUSE	CACAGTCTGA	CTTCAGATAT	ACTGTCTTTT TCCTGGCTCC TTGGGCTTAG	
CATTLE	AGCAGCCTGG	CTTCAGATT.	.CTGGCCTCT GCTT...T..	
GOAT	AGCAGCCTGG	CTTCAGATT.	.CTGGCCTCT GCTTGGCT..	
HUMAN	TGCAGCCTGG	CTTCAGATA.	.CTGGCTTCT GCCTGGCTCC TTG.....A	
CONSENSUS	-GCAGCCTGG	CTTCAGATAT	-CTGGCTTCT GCCTGGCTCC TTGGG--TA-	
	232	MNF-1	NF-1	281
RAT	TTCCACCTTG	TCCTTGCCCA	GGTCCAAGAA AAAGCCCAGA ACCTTGGCAC	
MOUSE	GTCTACCTTG	TCCTTGCCCA	GGTCCAAGAA AAGGCCCAGA ACCTTGGCAC	
CATTLE	..CCACTTTG	TGCTTTTCAA	TGACCAAGAA .AAGCCCAGG CACTTGGAAAT	
GOAT	..CCACTTTG	TGCTTTTCAA	TGACCAAGAA .AATCCCAGG CCCTTGGAAAT	
HUMAN	TCCCACCTTG	CCCTTGTCAG	TGACCAAGAA GAAGCCCAGC ACCTTGGCAC	
CONSENSUS	TTCCACCTTG	TCCTTGTCAA	TGACCAAGAA AAAGCCCAG- ACCTTGGCAC	

	282	MYB		TCF-1	331
RAT	TGCTTTGCCA	GTTAATGTCT	AACCGAGGAA	TGTCTTGCTG	CCAAAAGGTG
MOUSE	TGTTTTGCCA	GTTAATGTCT	AACTGAGGAA	TGTCTTGCTG	CCAAAAGGTG
CATTLE	TTTTTACCCA	GTTAATTTCT	AACTAAAGAA	CCTCTCGTTG	CCAAAAGATA
GOAT	TGTTTACTCA	GTTAATTTCT	AACTAAAGAA	CCTCTTGTTG	CCAAAAGGTA
HUMAN	TGCTTTCCCA	GTTAATTTCT	AACTATGGAA	TCTCTTGCTG	TTAGAAGGTG
CONSENSUS	TGTTTTCCCA	GTTAATTTCT	AACTAAGGAA	TCTCTTGCTG	CCAAAAGGTG

	332	FI-FII		GR		CF1		DEF	381
RAT	.CAAACAGAG	ACCTTGATT	TCCAGGCACA	GGTGTGACCC	C	CAATGTCA			
MOUSE	.AAAACAGAG	ACCTTGATT	TCCAGGCACA	GGTGTGACCC	C	AATGTCA			
CATTLE	TAAAACAGAG	CCCTTGTAAC	TCTGGGCACA	ACTGTGACCC	C	AGTGTCA			
GOAT	TAAAACAGAG	CCCTTGTAAC	TGTGGGCACA	GCTGTGACCC	C	CATGTCA			
HUMAN	CGAAACAGTG	ACCTTGATT	TCCGGGCACA	GGTGTGACCC	CC	CAATGTCA			
CONSENSUS	TAAAACAGAG	ACCTTGATT	TCCGGGCACA	GGTGTGACCC	C	CAATGTCA			

	382	SBF-1		NF-AT3		GR		431
RAT	ATCATTTCCT	GTCTCTAACT	ACCAGAGGAA	AAACTAACAA	CAACAGCCTC			
MOUSE	ATCATTT..T	GTGTCTAACT	CCCAGGGGAA	AAACTAACAA	CAACAGACTC			
CATTLE	ATCATTTGGG	GTCTCTACCT	ATTAG GGAA AA	GAACAA	CAACCACCTC			
GOAT	ATCATTTGGG	GTCTCTACCT	ATTAG GGAA AA	GAACAA	CANCCACCTC			
HUMAN	ATCATTTGGG	GTCTCTAGCT	ATTA GGAA AA	AGAACAA	CAACAACCTC			
CONSENSUS	ATCATTTGGG	GTCTCTA-CT	ATTAG-GGAA	AAAAGAACAA	CAACAACCTC			

	432	TCF-1		C-ETS-2		481
RAT	ATGGTTTGGA	AAAGGTGAAC	TCTATGCCAA	ATGGGAAGAA	AAATTCTGAC	
MOUSE	ATGGCTTGGA	AAAGGTGAAT	TCTATGCCAA	AAGGGAAGGA	AAGTTCT.AC	
CATTLE	ACAGCCTAGA	AAAGGAAAAC	ACTGTGTCAA	AAGGGAAGAA	TATTCC..AC	
GOAT	ACAGCCTANA	AAAGGAAAAC	ACTGTGTCAA	AAGGGAAGAA	TATTCC..AC	
HUMAN	ACAGCTTGGA	CAAGGCAAAC	ATTATGCCAG	GAGGGAAGAA	TATTCC..AC	
CONSENSUS	ACAGCTTGGA	AAAGG-AAAC	ACTATGCCAA	AAGGGAAGAA	TATTCCCT-AC	

	482		HSTF		HSF2	TFII-I	531
RAT	CCCCACAGAA	ACAATCTCAA	GAGGCAGAAG	CAGAGAATAA	TTGGAGG.GA		
MOUSE	CCCCACAGAA	ACAATCTCAG	AGGGCAGAAG	CAGAGAATAA	TCTGAGG.GA		
CATTLE	CCCCATTAAA	ATA..ATTAA	GAAAACAGAAC	CAGAGGATCA	TTGGAGGAAA		
GOAT	CCCCATTAAA	ATA..ATTAA	GAAAACAGAAC	CAGAGGATCA	TTGGAGGAGA		
HUMAN	CCCCAAGAAA	ACAATATCAA	AAAAACAGAAC	TAGAGACTAA	TTGGAGGAGA		
CONSENSUS	CCCCA--AAA	ACAATATCAA	GAAAACAGAAC	CAGAGAATAA	TTGGAGGAGA		

	532	CAC-BP	SP1	TFIID		581
RAT	GAGGGCCAGC	CAAGGGCAGA	CATATATATA	TATATATTGA	TCACAGGCAC	
MOUSE	GAGGGCCAGC	CAAGGGCAGG	CAAGTATATA	TTGA	TCACAGGCAC	
CATTLE	GACTGCCAGT	GGGGGACAGA	TGTATATATA	TAGATATGAT	AGTCACCTAC	
GOAT	GATTGCCAGT	GGGGGACAGA	TGTATATATA	TAGATATGAA	AGTCACCTAC	
HUMAN	GATTGCCAGC	CTGGGGCAAA	TGTGTATATA	TAAGTATGAG	GCACA.....	
CONSENSUS	GA-TGCCAGC	C-GGGGCAGA	TGTATATATA	TA-ATATGAA	-CACA---AC	

	582	591
RAT	TTACTTGTGA	
MOUSE	TTACTTGTGA	
CATTLE	TTGTAAAAGG	
GOAT	TTGTAAAAGG	
HUMAN	TCATCACCAG	
CONSENSUS	TTAT-A--GG	

Sequence Identity of Uromodulin Promoters of Different Mammals

	Mouse	Rat	Goat	Cattle	Human
Mouse	N/C	90%	74%	73%	77%
Rat	90%	N/C	73%	74%	73%
Goat	74%	73%	N/C	94%	76%
Cattle	73%	74%	94%	N/C	78%
Human	77%	73%	76%	78%	N/C

Identity between sequence pairs are indicated as percentage identity. Note that promoters of two farm animals, goat and cattle, share ⁹⁴100% identity.

N/C: Sequences from the same species are 100% identical and are thus not compared.

Mouse versus rat

BestFit Results



BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092

REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092

February 18, 2001 21:38

(No documentation)

to: rat589 check: 5372 from: 1 to: 581

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000

Quality:	4177	Length:	585
Ratio:	7.315	Gaps:	8
Percent Similarity:	90.459	Percent Identity:	90.459

Match display thresholds for the alignment(s):

IDENTITY

$$1 = 5$$

. = 1

m.THP.promoter.log x rat589 September 22, 2004 10:34 ..

```

1522 AGTCTTGCTGTGACAGAGGTCAGTTCCAGTAGTGTCCAGATGGTCTGATAA 1571
      |||||
2 agtcttgctgtgacagaggtccagttgagggatgtccagatggctcttgcaa 51

1572 CCTGATGCCATTCTCAGAGA...CTCTTTCCTGTCTGGAATCTAGTGAG 1617
      ||| ||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
52 cc.gataactttctcagagactctctcttttcctgtctggactctagtggg 100

1618 GAGGACTTATCTGGTGAAGCTGTCCTTTAGAACAGGAGTGTGTTCCAGTC 1667
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
101 gaggactaatctggtgaagctgttcttcagatcaggtgtgtgttccaggc 150

1668 TTCAAAGCAAACATTCTCTTTTATCCTAACACAGTCTGACTTCAGATATAC 1717
      ||| ||||| ||| ||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
151 ttcgaagcaaatgtttctgttatcctaaccaggctggcttcagatat.. 198

1718 TGTCTTTTTCTGGCTCCTTGGGCCTTAGGTCTACCTTGTCTCTTGCCCAGG 1767
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
199 tgtcttttttctgcccccttgg..tatttccaccttgtccttgcccagg 246

1768 TCCAAGAAAAGGCCCAGAACCTTGGCACTGTTTTGCCAGTTAATGTCTAA 1817
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
247 tccaagaaaaagcccagaaccttggcactgctttgccagttaatgtctaa 296

1818 CTGAGGAATGTCTTGCTGCCAAAAGGTGAAAACAGAGACCTTGTATTTCC 1867
      | ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

```

Input Sequence: m.THP.promoter.log

View Sequence

Input Sequence: rat589

!!NA_SEQUENCE 1.0

rat589 Length: 581 September 18, 2001 10:45 Type: N
Check: 5372 ..

1 tagtcttgtc tgacagaggt ccagttgagg gatgtccaga
tggctcttgca

51 accgataact ttctcagaga ctctctcttt cctgtctgga
ctctagtggg

[View Sequence](#)

*Mouse versus goat***BestFit Results**BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092

February 18, 2001 21:38

(No documentation)

to: g.THP.promoter18.log check: 4545 from: 1 to: 1630REFORMAT of: g.THP.promoter18.log.22665 check: 4545 from: 1 to: 1630

February 18, 2001 21:37

(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000

Quality:	1374	Length:	422
Ratio:	3.444	Gaps:	11
Percent Similarity:	74.300	Percent Identity:	73.791

Match display thresholds for the alignment(s):

	=	IDENTITY
:	=	5
.	=	1

m.THP.promoter.log x g.THP.promoter18.log September 22, 2004 10:32 ..

```

1677 AACATTCCTTTTATCCTAACACAGTCTGACTTCAGATATACTGTCTTTTT 1726
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1121 AGCACCCCTTTTCTCCTGGAGCAGCCTGGCTTCAGA.....T 1157

1727 CCTGGCTCCTTGGGCTTAGGTCTACCTTGTCTTGCCAGGTCCAAGAAA 1776
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1158 TCTGGCCTCT...GCTTGGCTCCACTTTGTGCTTTTCAATGACCAAGAAA 1204

1777 AGGCCCAGAACCTTGGCACTGTTTTGCCAGTTAATGTCTAACTGAGGAAT 1826
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1205 A.TCCCAGGCCCTTGAATTGTTTACTCAGTTAATTTCTAACTAAAGAAC 1253

1827 GTCTTGCTGCCAAAAGGT.GAAAACAGAGACCTTGTATTTCCAGGCACAG 1875
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1254 CTCTTGTTGCCAAAAGGTATAAAACAGAGCCCTTGTAGCTGTGGGCACAG 1303

1876 GTGTGACCCCCAATGTCAATCATTT..TGTGTCTAACTCCCAGGGGAAAAA 1923
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1304 CTGTGACCCCCATGTCAATCATTTGGGGTCTCTACCTATTAGGG...AAA 1350

1924 CTAACAACAACAGACTCATGGCTTGGAAAAGGTGAATTCTATGCCAAAAG 1973
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1351 AGAACAACANCCACCTCACAGCCTANAAAAGGAAAACACTGTGTCAAAAAG 1400

```

```
1974 GGAAGGAAAGTTCTACCCCCACAGAAACAATCTCAGAGGGCAGAAGCAGA 2023
      ||||  ||  ||| ||||| ||| ||| |||  ||||| |||
1401 GGAA.AAATATTCCACCCCATTAAATAAT.TAAGA.AACAGAACCAGA 1447
      |
2024 GAATAATCTGAGG.GAGAGGGCCAGCCAAGGGCAG..GCAAGTATATATT 2070
      ||| ||| ||||| ||||| ||| ||| |||||
1448 GGATCATTGGAGGAGAGATTGCCAGTGGGGGACAGATGTATATATATAGA 1497
      |
2071 GATCACAGGCACTTACTTGTGA 2092
      || | || ||| ||||| |||
1498 TATGAAAGTCACCTACTTGTAA 1519
```

Input Sequence: m.THP.promoter.log

```
!!NA_SEQUENCE 1.0
  REFORMAT of: m.THP.promoter.log.29746  check: 5833  from: 1
to: 2092  February 18, 2001 21:38
```

(No documentation)

```
m.THP.promoter.log  Length: 2092  February 18, 2001 21:38
Type: N  Check: 5833  ..
```

```
1  AATTTAAGAC TGGTTGATTT CTTGAATTC AGTGGGCTTG
```

[View Sequence](#)

Input Sequence: g.THP.promoter18.log

```
!!NA_SEQUENCE 1.0
  REFORMAT of: g.THP.promoter18.log.22665  check: 4545  from:
1  to: 1630  February 18, 2001 21:37
```

(No documentation)

```
g.THP.promoter18.log  Length: 1630  February 18, 2001 21:37
Type: N  Check: 4545  ..
```

```
1  ACTATAGGGC ACGCGTGGTC GACGGCCCGG GCTGGTAAAG
```

[View Sequence](#)

*Mouse versus cattle***BestFit Results**BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092

February 18, 2001 21:38

(No documentation)

to: cattle.THP.promoter.txt check: 7177 from: 1 to: 626REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177 from: 1 to: 626

February 18, 2001 21:37

(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000

Quality:	1284	Length:	439
Ratio:	3.109	Gaps:	12
Percent Similarity:	72.973	Percent Identity:	72.973

Match display thresholds for the alignment(s):

	=	IDENTITY
:	=	5
.	=	1

m.THP.promoter.log x cattle.THP.promoter.txt September 22, 2004 10:26 ..

```

1660 TTCCAGTCTTCAAAGCAAACATTCCTTTTATCCTAACACAGTCTGACTTC 1709
    | ||||| | || | || | ||||| ||| ||| |||||
168  tcccagtggggacagtgagcacccttttctcctggagcagcctggcttc 217

1710 AGATATACTGTCTTTTTCCTGGCTCCTTGGGCTTAGGTCTACCTTGTCTCT 1759
    ||| | ||||| || | ||| | ||| ||||| ||
218  aga.....ttctggcctct...gctt...tccactttgtgct 248

1760 TGCCCAGGTCCAAGAAAAGGCCCAAGAACCTTGGCACTGTTTTGCCAGTTA 1809
    | | | | ||||| ||||| ||||| | | | | |||||
249  tttcaatgaccaagaaaa.gcccaggcacttggaattttttaccagtta 297

1810 ATGTCTAACTGAGGAATGTCTTGCTGCCAAAAGGT.GAAAACAGAGACCT 1858
    || ||||| || | || | ||||| | ||||| ||||| |||
298  atttctaactaaagaacctctcggttgccaaaagatataaaacagagccct 347

1859 TGTATTTCCAGGCACAGGTGTGACCCCAATGTCAATCATTT..TGTGTCT 1906
    |||| | | ||||| ||||| ||||| ||||| ||||| |||
348  tgtaactctgggcacaaactgtgacccagtgatcaatcatttggggctctct 397

1907 AACTCCCAGGGGAAAAAATAACAACAACAGACTCATGGCTTGGAAAAGGT 1956
    | | | |||| | || | ||||| |||| | | | |||||
398  acctattaggg...aaaagaacaacaaccacctcacagcctagaaaagga 444

```

Input Sequence: m.THP.promoter.log

1 AATTTAAGAC TGGTTGATT CTTGAATTC AGTGGGCTTG

View Sequence

1 aatttcttga ttcacagagc atctggtcca atgatgtctg

View Sequence

*Mouse versus human***BestFit Results**BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092

February 18, 2001 21:38

(No documentation)

to: human.THP.promoter.txt check: 7451 from: 1 to: 620REFORMAT of: human.THP.promoter.txt.30475 check: 7451 from: 1 to: 620

February 18, 2001 21:36

(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000

Quality:	1790	Length:	407
Ratio:	4.509	Gaps:	7
Percent Similarity:	76.590	Percent Identity:	76.590

Match display thresholds for the alignment(s):

	=	IDENTITY
:	=	5
.	=	1

m.THP.promoter.log x human.THP.promoter.txt September 22, 2004 10:33 ..

```

1682 TCCTTTTATCCTAACACAGTCTGACTTCAGATATACTGTCTTTTCTCTGG 1731
      ||||| |||| | ||| || ||||| ||||| ||| |||||
173 tccttttgccttatgcagcctggcttcag..atactggcttctgcctgg 220

1732 CTCCTTGGGCTTAGGTCTACCTTGTCTTGGCCAGGTCCAAGAAAAGGCC 1781
      ||||| | | ||| || ||||| | | ||||| ||| |||
221 ctccttgatc.....ccaccctgcccttgctcagtgaccaagaagaagcc 264

1782 CAGAACCTTGGCACTGTTTGGCCAGTTAATGTCTAACTGAGGAATGTCTT 1831
      ||| ||||| ||||| ||| ||||| ||||| ||||| ||| |||
265 cagcaccttggcactgctttcccagttaatttctaactatggaatctctt 314

1832 GCTGCCAAAAGGTG.AAAACAGAGACCTTGTATTTCCAGGCACAGGTGTG 1880
      |||| | ||||| ||||| ||||| ||||| ||||| |||||
315 gctgttagaaggtgcgaaacagtgaccttgatttccgggcacaggtgtg 364

1881 A..CCCCAATGTCAATCATTTTGTGTCTAACTCCCAGGGGAAAAACTAAC 1928
      | ||||| ||||| ||||| ||||| | ||||| ||| |||
365 accccccaatgtcaatcatttggggtctctagctattaggaaaaa.gaac 413

1929 AACACAGACTCATGGCTTGGAAGGTGAATTCTATGCCAAAAGGGAAG 1978
      ||||| |||| ||||| |||| || ||||| ||| |||||
414 aacaacaacctcacagcttgacaaggcaaacattatgccagga.ggaaa 462

```

```
1979 GAAAGTTCTACCCCCACAGAAACAATCTCAGAGGGCAGAAGCAGAGAATA 2028
    || ||| ||||| ||||| ||| | |||| ||||| ||
463 aaatattccaccccccaagaaaacaatatcaaaaaacagaactagagacta 512

2029 ATCTGAGG.GAGAGGGCCAGCCAAGGGCAGGCAAGTATATATTGATCACA 2077
    || ||| |||| ||||| ||||| ||||| ||| |
513 attggaggagagattgccagcctggggcaaatgtgtatatataagtatga 562

2078 GGCACCTT 2084
    ||||| |
563 ggcacat 569
```

Input Sequence: m.THP.promoter.log

```
!!NA_SEQUENCE 1.0
  REFORMAT of: m.THP.promoter.log.29746  check: 5833  from: 1
to: 2092  February 18, 2001 21:38

  (No documentation)

m.THP.promoter.log  Length: 2092  February 18, 2001 21:38
Type: N  Check: 5833  ..

      1  AATTTAAGAC TGGTTGATTT CTTGAATTTT AGTGGGCTTG
```

[View Sequence](#)

Input Sequence: human.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
  REFORMAT of: human.THP.promoter.txt.30475  check: 7451
from: 1  to: 620  February 18, 2001 21:36

  (No documentation)

human.THP.promoter.txt  Length: 620  February 18, 2001 21:36
Type: N  Check: 7451  ..

      1  cagagtgggt caggtccagt gatgtctgaa ctaccttctg
```

[View Sequence](#)

BestFit Results

Rat versus goat

Reine

BESTFIT of: Rat.THP.promoter.txt check: 1164 from: 1 to: 625

REFORMAT of: Rat.THP.promoter.txt.27903 check: 1164 from: 1 to: 625
February 18, 2001 21:37
(No documentation)

to: g.THP.promoter18.log check: 4545 from: 1 to: 1630

REFORMAT of: g.THP.promoter18.log.22665 check: 4545 from: 1 to: 1630
February 18, 2001 21:37
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight: 50 Average Match: 10.000
Length Weight: 3 Average Mismatch: -9.000

Quality: 1640 Length: 462
Ratio: 3.744 Gaps: 9
Percent Similarity: 73.733 Percent Identity: 73.272

Match display thresholds for the alignment(s):

| = IDENTITY
: = 5
. = 1

Rat.THP.promoter.txt x g.THP.promoter18.log September 22, 2004 11:11 ..

```

168 ctgttatcctaaccaggctggccttcagatattgtcttttttctgcccc 217
   || || ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
1128 CTTTTCTCCTGGAGCAGCCTGGCTTCAGA.....TTCTGGCCT 1165

218 tttggtatttccaccttgctccttgcccagggtccaagaaaaagcccagaac 267
   | | | | | | | | | | | | | | | | | | | | | | | |
1166 CTGCTTGGCTCCACTTTGTGCTTTTCAATGACCAAG.AAAATCCCAGGCC 1214

268 cttggcactgctttgcccagttaatgtctaaccgaggaatgtcttgctgcc 317
   ||||| ||| ||| ||||| ||||| ||||| ||||| ||||| |||||
1215 CTTGGAATTGTTTACTCAGTTAATTCTAACTAAAGAACCTCTTGTTGCC 1264

318 aaaagggt.gcaaacagagaccttgatatttcaggcacagggtgtgaccccc 366
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1265 AAAAGGTATAAAACAGAGCCCTTGTAGCTGTGGGCACAGCTGTGACCCC 1314

367 aatgtcaatcattttctgtctctaactaccagaggaaaaaactaacaacaa 416
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1315 .ATGTCAATCATTTGGGGTCTCTACCTATTAGGG...AAAAGAACAACAN 1360

417 cagcctcatgggttggaagggtgaactctatgccaaatgggaagaaaaa 466
   | ||||| | | : ||||| ||| ||| ||| ||| ||| ||| |||
1361 CCACCTCACAGCCTANAAAAGGAAAACACTGTGTCAAAAGGGAAAAATAT 1410

```


Rat versus cattle

BestFit Results

BESTFIT of: Rat.THP.promoter.txt check: 1164 from: 1 to: 625REFORMAT of: Rat.THP.promoter.txt.27903 check: 1164 from: 1 to: 625

February 18, 2001 21:37

(No documentation)

to: cattle.THP.promoter.txt check: 7177 from: 1 to: 626REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177 from: 1 to: 626

February 18, 2001 21:37

(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000

Quality:	1598	Length:	461
Ratio:	3.682	Gaps:	10
Percent Similarity:	74.186	Percent Identity:	74.186

Match display thresholds for the alignment(s):

| = IDENTITY

: = 5

. = 1

Rat.THP.promoter.txt x cattle.THP.promoter.txt September 22, 2004 11:13 ..

```

168 ctgttatcctaaccaggctggcttcagatattgtcttttttctgcccc 217
    || || ||| ||| ||||| ||| ||| ||| ||| ||| |||
192 cttttctcctggagcagcctggcttcagattctggcct.....ctgc... 233
218 tttggtatttccacctgtctcctgcccagggtccaagaaaaagcccagaac 267
    ||||| ||| ||| ||| ||| ||| ||| ||| ||| |||
234 .....ttccactttgtgcttttcaatgaccaag.aaaagcccaggca 275
268 cttggcactgctttgccagttaatgtctaaccgaggaatgtcttgctgcc 317
    ||||| ||| ||| ||||| ||||| ||||| ||| ||| ||| |||
276 cttggaattttttaccagttaatttctaactaagaacctctcgttgcc 325
318 aaaaggt.gcaaacagagacctgtatttccaggcacagggtgtgaccccc 366
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
326 aaaagatataaaacagagcccttgtaactctgggcacaactgtga.cccc 374
367 aatgtcaatcattttctgtctctaactaccagaggaaaaactaacaacaa 416
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
375 agtgtcaatcatttggggtctctacctattagg...aaaagaacaacaa 421
417 cagcctcatggttttgaaaagggtgaactctatgccaaatgggaagaaaaa 466
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
422 ccacctcacgcctagaaaaggaaaacactgtgtcaaaagggaaaaaatat 471

```

```

467 ttctgacccccacagaaacaatctcaagaggcagaagcagagaataattg 516
   || ||||| ||| || ||| ||||| ||||| |||||
472 tcc..acccccattaaaataa..ttaagaaacagaaccagaggatcattg 517
   || ||||| ||| || ||| ||||| ||||| |||||
517 gagggagagggccagccaagggcagacatatatatatatattgatcac 566
   || ||||| ||| || ||| ||||| ||||| |||||
518 gag...gaaagactgccagtgggggacagatgtatatatatagatatgat 564
   || ||||| ||| || ||| ||||| ||||| |||||
567 aggcacttacttgtgaatggaccagtcct...gtcctgggttcaggtaag 613
   || ||||| ||||| || ||| ||| ||| ||||| |||||
565 agtcacctacttgtaaaaggattaattctacctttctggtttcaggtaag 614
   || ||||| ||| || ||| ||||| ||||| |||||
614 actgtctggag 624
   || ||||| |||
615 gctatctgcag 625

```

Input Sequence: Rat.THP.promoter.txt

```

!!NA_SEQUENCE 1.0
REFORMAT of: Rat.THP.promoter.txt.27903  check: 1164  from:
1  to: 625  February 18, 2001 21:37

(No documentation)

Rat.THP.promoter.txt  Length: 625  February 18, 2001 21:37
Type: N  Check: 1164  ..

1  ctagtcttgt ctgacagagg tccagttgag ggatgtccag

```

[View Sequence](#)

Input Sequence: cattle.THP.promoter.txt

```

!!NA_SEQUENCE 1.0
REFORMAT of: cattle.THP.promoter.txt.27851  check: 7177
from: 1  to: 626  February 18, 2001 21:37

(No documentation)

cattle.THP.promoter.txt  Length: 626  February 18, 2001
21:37  Type: N  Check: 7177  ..

1  aatttcttga ttcacagagc atctgggtcca atgatgtctg

```

[View Sequence](#)

*Rat vers us human***BestFit Results**BESTFIT of: Rat.THP.promoter.txt check: 1164 from: 1 to: 625

REFORMAT of: Rat.THP.promoter.txt.27903 check: 1164 from: 1 to: 625

February 18, 2001 21:37

(No documentation)

to: human.THP.promoter.txt check: 7451 from: 1 to: 620

REFORMAT of: human.THP.promoter.txt.30475 check: 7451 from: 1 to: 620

February 18, 2001 21:36

(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000

Quality:	2040	Length:	588
Ratio:	3.611	Gaps:	10
Percent Similarity:	73.297	Percent Identity:	73.297

Match display thresholds for the alignment(s):

	=	IDENTITY
:	=	5
.	=	1

Rat.THP.promoter.txt x human.THP.promoter.txt September 22, 2004 11:14 ..

```

60 ctttctcagagactctctcttttctgtctggactctagtggggaggacta 109
   | ||||| | ||||| | ||||| | ||||| | |||||
56 cattctcagctcctctcttgccttggtctggattct.....aaggctg 98
   | ||||| | ||||| | ||||| | ||||| | |||||
110 atctggtgaagctg.....ttcttcagatcaggtgt 140
    |||| | || | || ||||| | |||||
99 atctcatgagaatgggtgtttcagaagggtgccctctccaagacaggtgc 148
   | |||| | || | || ||||| | |||||
141 gtgttccaggcttcgaagcaaagtgttctgttatcctaaccaggctggc 190
    | || | | || | || | || ||||| | |||||
149 acctcccatctggggcagtgat.atccttttgtccttatgcagcctggc 197
   | |||| | || | || ||||| | |||||
191 ttcagatatgtcttttttctgccccttggtatttccacctgtcctt 240
    ||||| | || | || ||||| | |||||
198 ttcagatactggcttctgcctggctcct....tgatcccacctgcccct 243
   | |||| | || | || ||||| | |||||
241 gcccagggtccaagaaaaagcccagaaccttggcactgctttgccagttaa 290
    | | | ||||| | ||||| | ||||| | |||||
244 gtcagtgaccaagaagaagcccagcaccttggcactgctttcccagttaa 293
   | |||| | || | || ||||| | |||||
291 tgtctaaccgaggaatgtcttgctgccaaaagggtgc.aaacagagacctt 339
    | |||| | || | || ||||| | |||||
294 tttctaactatggaatctcttgctgttagaagggtgcgaaacagtgacctt 343

```

```

340 gtattttccaggcacaggtgtga.cccccaatgtcaatcattttctgtctc 388
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
344 gtattttccgggcacaggtgtgaccccccaatgtcaatcatttgggggtctc 393
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
389 taactaccagaggaaaaaactaacaacaacagcctcatggtttggaaaagg 438
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
394 tagctatta...ggaaaaagaacaacaacacctcacagcttggaacaagg 440
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
439 tgaactctatgccaaatgggaagaaaaattctgacccccacagaaacaat 488
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
441 caaacattatgccaggaggaaaaaatattcc..acccccaagaaaacaat 488
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
489 ctcaagaggcagaagcagagaataattggagg.gagagggccagccaagg 537
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
489 atcaaaaaacagaactagagactaattggaggagagattgccagcctggg 538
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
538 gcagacatatatatatatattgatcacaggcacttacttgtgaatgga 587
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
539 gcaaattgtgtatatataagtat.....gaggcacatcatcaccagacta 582
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
588 ccagtctgtctctgggttcaggtaagactgtctggagc 625
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
583 actctacctttctgggttcaggtaaggctatctgtagc 620

```

Input Sequence: Rat.THP.promoter.txt

```

!!NA_SEQUENCE 1.0
REFORMAT of: Rat.THP.promoter.txt.27903  check: 1164  from:
1  to: 625  February 18, 2001 21:37

(No documentation)

Rat.THP.promoter.txt  Length: 625  February 18, 2001 21:37
Type: N  Check: 1164  ..

1  ctagtcttgt ctgacagagg tccagttgag ggatgtccag

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[View Sequence](#)

Input Sequence: human.THP.promoter.txt

!!NA_SEQUENCE 1.0

REFORMAT of: human.TH.Promoter.txt.30475 check: 7451

from: 1 to: 620 February 18, 2001 21:36

(No documentation)

human.TH.Promoter.txt Length: 620 February 18, 2001 21:36

Type: N Check: 7451 ..

1 cagagtgggt caggtccagt gatgtctgaa ctaccttctg

[View Sequence](#)

*goat versus cattle***BestFit Results****Refine**BESTFIT of: g.THP.promoter18.log check: 4545 from: 1 to: 1630REFORMAT of: g.THP.promoter18.log.22665 check: 4545 from: 1 to: 1630

February 18, 2001 21:37

(No documentation)

to: cattle.THP.promoter.txt check: 7177 from: 1 to: 626REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177 from: 1 to: 626

February 18, 2001 21:37

(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000

Quality: 5545 Length: 628

Ratio: 8.872 Gaps: 1

Percent Similarity: 94.560 Percent Identity: 94.240

Match display thresholds for the alignment(s):

| = IDENTITY

: = 5

. = 1

g.THP.promoter18.log x cattle.THP.promoter.txt September 22, 2004 10:36 ..

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1  aatttcttgattcacagagcatctgggtccaatgatgtctgaattgccttc 50

987 TGTCTCTGACCTTCAGCCATTCTCAGCTCCTTCTGATCACATTGGGAC 1036
    |||
51 tgtctctgaccttcaggcattctcagctcctttctgctcacatcgggac 100

1037 CCCAGGGGAGCTGGCTGAATCTGTGAGGATGGCATTGCTTTGGAATTAA 1086
    |||
101 cccaggaagctggttgaacccatgaggatggaacttgctttggaactga 150

1087 GTGGCCACAAGTACACATCCTGGTGGGGACGATGAGCACCCCTTTTCTCC 1136
    |||
151 gtggccacaagtatacatcccagtggggacagtgagcacccttttctcc 200

1137 TGGAGCAGCCTGGCTTCAGATTCTGGCCTCTGCTTGGCTCCACTTTGTGC 1186
    |||
201 tggagcagcctggcttcagattctggcctctgctt...tccactttgtgc 247

1187 TTTTCAATGACCAAGAAAATCCCAGGCCCTTGGAATTGTTTACTCAGTTA 1236
    |||
248 ttttcaatgaccaagaaaagcccaggcacttggaattttttaccagtta 297

```

```

1237 ATTTCTAACTAAAGAACCTCTTGTTGCCAAAAGGTATAAAACAGAGCCCT 1286
      |||
298 atttctaactaaagaacctctcgttgccccaaagatataaaacagagccct 347
      |||
1287 TGTAGCTGTGGGCACAGCTGTGACCCCCATGTCAATCATTGTTGGGGTCTCT 1336
      |||
348 tgtaactctgggcacaaactgtgacccccagtgtcaatcatttgggtctct 397
      |||
1337 ACCTATTAGGGAAAAGAACAACANCCACCTCACAGCCTANAAAAGGAAAA 1386
      |||
398 acctattagggaaaagaacaacaaccacctcacagcctagaaaaggaaaa 447
      |||
1387 CACTGTGTCAAAAGGGAAAAATATTCCACCCCCATTAAAAATAATTAAGAA 1436
      |||
448 cactgtgtcaaaagggaaaaatattccacccccattaaaataattaagaa 497
      |||
1437 ACAGAACCAGAGGATCATTGGAGGAGAGATTGCCAGTGGGGGACAGATGT 1486
      |||
498 acagaaccagaggatcattggaggaaagactgccagtgggggacagatgt 547
      |||
1487 ATATATATAGATATGAAAGTCACCTACTTGTAAGGATTAATTCTACCT 1536
      |||
548 atatatatagatatgatagtcacctacttgtaaaaggattaattctacct 597
      |||
1537 TTCTGGTTTCAGGTAAGGCTATCTGCAG 1564
      |||
598 ttctggtttcaggtaaggctatctgcag 625

```

Input Sequence: g.THP.promoter18.log

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!!NA_SEQUENCE 1.0
REFORMAT of: g.THP.promoter18.log.22665  check: 4545  from:
1  to: 1630  February 18, 2001 21:37

(No documentation)

g.THP.promoter18.log  Length: 1630  February 18, 2001 21:37
Type: N  Check: 4545  ..

1  ACTATAGGGC ACGCGTGGTC GACGGCCCCG GCTGGTAAAG

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[View Sequence](#)

Input Sequence: cattle.THP.promoter.txt

!!NA_SEQUENCE 1.0
REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177
from: 1 to: 626 February 18, 2001 21:37

(No documentation)

cattle.THP.promoter.txt Length: 626 February 18, 2001
21:37 Type: N Check: 7177 ..

1 aatttcttga ttcacagagc atctgggtcca atgatgtctg

[View Sequence](#)

Goat versus human

Refine

BESTFIT of: g.THP.promoter18.log check: 4545 from: 1 to: 1630

```

REFORMAT of: g.THP.promoter18.log.22665  check: 4545  from: 1  to: 1630
February 18, 2001 21:37
(No documentation)

```

to: human.THP.promoter.txt check: 7451 from: 1 to: 620

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REFORMAT of: human.THP.promoter.txt.30475  check: 7451  from: 1  to: 620
February 18, 2001 21:36
(No documentation)

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Symbol comparison table: swgapdna.cmp CompCheck: 2335

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Length Weight:	3	Average Mismatch:	-9.000

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Percent Similarity: 76.325  Percent Identity: 75.993

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Match display thresholds for the alignment(s):

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. = 1

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g.THP.promoter18.log x human.THP.promoter.txt September 22, 2004 11:22 ..

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949 CACAGAGCATCTGGTCCAATGATGTCTGAATTATCTTGCTGTCTCTGACCT 998
    ||||| | ||||||| ||||||| ||||| | |||||
   1 cagagtgggtcagggtccagtgatgtctgaactaccttctggttctgactt 50

999 TCAGCCATTCTCAGCTCCTTTCTGATCACATTGGGACCCCAGGGGAGCT 1048
    ||||||| ||||||| ||||| | ||||| |
   51 tcagccattctcagctcctctcttgccttgtgtctggattcta.....a 93

1049 GGCTGAATCTGTGAGGATGGCATTGTCTTTGGAATTAAGTGGCC...ACA 1095
    |||||| | ||||| ||||| ||||| | ||||| | |||||
   94 ggctgatctcatgagaatgggtgtttcagaaggggtgccctctccaagaca 143

1096 AGTACACATCCTGGTGGGGACGATGAGCACCCCTTTTCTCCTGGAGCAGC 1145
    ||||| ||||| ||||| ||||| ||||||| ||||| |||||||
  144 ggtgcacctcccactctggggcagtgaaata.tccttttgccttatgcagc 192

1146 CTGGCTTCAGATTCTGGCCTCTGCTTGGCT.....CCACTTTGTGCT 1187
    ||||||| ||||||| ||||||| ||||| ||||| ||||| |||||
  193 ctggcttcagatactggcttctgcctggctccttgatcccaccctgacct 242

1188 TTTCAATGACCAAGAA.AATCCCAGGCCCTTGAATTGTTTACTCAGTTA 1236
    ||||| ||||||| ||||| ||||||| ||||||| ||||||| |||||||
  243 tgtcagtgaccaagaagaagcccagcaccttggcactgctttccagtta 292

```

Input Sequence: g.THP.promoter18.log

View Sequence

9/22/2004

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!!NA_SEQUENCE 1.0
REFORMAT of: human.THP.promoter.txt.30475  check: 7451
from: 1  to: 620  February 18, 2001 21:36

(No documentation)

human.THP.promoter.txt  Length: 620  February 18, 2001 21:36
Type: N  Check: 7451  ..

      1  cagagtgggt caggtccagt gatgtctgaa ctaccttctg
```

[View Sequence](#)


```

293 agttaatttctaactaaagaacctctcggttgccaaaagatataaaacaga 342
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
288 agttaatttctaactatggaatctcttgctggttagaagggtgcgaaacagt 337

343 gcccttgtaactctgggcacaactgtga..ccccagtgtcaatcatttgg 390
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
338 gaccttgatattccgggcacaggtgtgaccccccaatgtcaatcatttgg 387

391 ggtctctacctattagggaaaagaacaacaaccacctcacagcctagaaa 440
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
388 ggtctctagctattagggaaaaagaacaacaacacacacagcttgga 437

441 aggaaaacactgtgtcaaaagggaataattccacccccattaaaata. 489
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
438 aggcaaacattatgccaggaggaaaaaataattccaccccccaagaaaaca 487

490 .attaagaaacagaaccagaggatcattggaggaaagactgccagtgggg 538
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
488 tatcaaaaaacagaactagagactaattggaggagagattgccagcctgg 537

539 gacagatgtatatatatagatatgatagtcacctacttgtaaaaggatta 588
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
538 ggcaaatgtgtatatataagtatgaggcaca.....tcatcaccagacta 582

589 attctacctttctgggtttcaggttaaggctatctgcag 625
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Input Sequence: cattle.THP.promoter.txt

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!!NA_SEQUENCE 1.0
REFORMAT of: cattle.THP.promoter.txt.27851  check: 7177
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(No documentation)

cattle.THP.promoter.txt Length: 626 February 18, 2001
21:37 Type: N Check: 7177 ..

1 aatttcttga ttcacagagc atctggtcca atgatgtctg

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[View Sequence](#)

Input Sequence: human.THP.promoter.txt

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!!NA_SEQUENCE 1.0
REFORMAT of: human.THP.promoter.txt.30475  check: 7451
from: 1  to: 620  February 18, 2001 21:36

(No documentation)

human.THP.promoter.txt  Length: 620  February 18, 2001 21:36
Type: N  Check: 7451  ..

      1  cagagtgggt caggtccagt gatgtctgaa ctaccttctg
```

[View Sequence](#)

A humane e lucif
constant

Tamm-Horsfall Glycoprotein: Biology and Clinical Relevance

Franca Serafini-Cessi, MD, Nadia Malagolini, PhD, and Daniela Cavallone, PhD

• Tamm-Horsfall glycoprotein (THP) is the most abundant urinary protein in mammals. Urinary excretion occurs by proteolytic cleavage of the large ectodomain of the glycosyl phosphatidylinositol-anchored counterpart exposed at the luminal cell surface of the thick ascending limb of Henle's loop. We describe the physical-chemical structure of human THP and its biosynthesis and interaction with other proteins and leukocytes. The clinical relevance of THP reported here includes: (1) involvement in the pathogenesis of cast nephropathy, urolithiasis, and tubulointerstitial nephritis; (2) abnormalities in urinary excretion in renal diseases; and (3) the recent finding that familial juvenile hyperuricemic nephropathy and autosomal dominant medullary cystic kidney disease 2 arise from mutations of the THP gene. We critically examine the literature on the physiological role and mechanism(s) that promote urinary excretion of THP. Some lines of research deal with the *in vitro* immunoregulatory function *in vivo* has not yet been established. In the most recent literature, there is renewed interest in the capacity of urinary THP to compete efficiently with urothelial cell receptors, such as uroplakins, in adhering to type 1 fimbriated *Escherichia coli*. This property supports the notion that abundant THP excretion in urine is promoted in the host by selective pressure to obtain an efficient defense against urinary tract infections caused by uropathogenic bacteria. *Am J Kidney Dis* 42: 658-676.

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INDEX WORDS: Tamm-Horsfall protein (THP); uromodulin; urinary proteins; glycosyl phosphatidylinositol (GPI)-anchored proteins; cast nephropathy; myeloma nephropathy; stone disease; interstitial nephritis; *Escherichia coli*; familial juvenile hyperuricemic nephropathy (FJHN); medullary cystic kidney disease 2 (MCKD2).

IN 1950, IGOR TAMM and Frank Horsfall¹ used a salt-precipitation procedure to isolate a potent inhibitor of viral hemoagglutination from urine of healthy individuals. Subsequently, the same investigators² undertook the chemical characterization of mucoprotein and the way in which it inhibits hemoagglutination induced by influenza, mumps, and Newcastle disease viruses. In general terms, the 2 investigators sought to obtain evidence of the (then) putative enzymatic activity associated with viruses and identify inhibitors preventing viruses from binding to susceptible cells. They reported that neuraminidase treatment abolished the inhibitory effect in the viral hemoagglutination assay. This observa-

tion persuaded Gottschalk³ and Odin⁴ to analyze the carbohydrate moiety of urinary mucoprotein. Both studies established that carbohydrate content accounts for more than 20% to 25% of mucoprotein weight, and sialic acid is abundantly present. Urinary mucoprotein has since become known as Tamm-Horsfall glycoprotein or Tamm-Horsfall protein (THP).

In 1964, Bayer⁵ used electron microscopy to confirm the binding of influenza virus to urinary mucoprotein. Urinary THP was visualized as a network of filaments composed of smaller fibrils with a diameter of 4 to 24 nm, but the length could not be detected because of their tendency to form end-to-end aggregates. In the 1970s, Albert Neuberger's laboratory in London showed that THP: (1) migrates in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a single molecular unit with an apparent molecular weight of 80 to 90 kd; (2) is the most abundant protein in normal urine, excreted at the rate of approximately 50 mg/d; and (3) is present in urine of other mammals.⁶⁻⁹

THP had been constantly under the attention of investigators working in the field of glycoproteins or nephrologists and urologists (according to PubMed from January 1967 to January 1985, almost 200 articles concerning Tamm-Horsfall glycoprotein were published) when, in 1985, it was rediscovered by Muchmore and Decker.¹⁰

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doi:10.1053/S0272-6386(03)00829-1

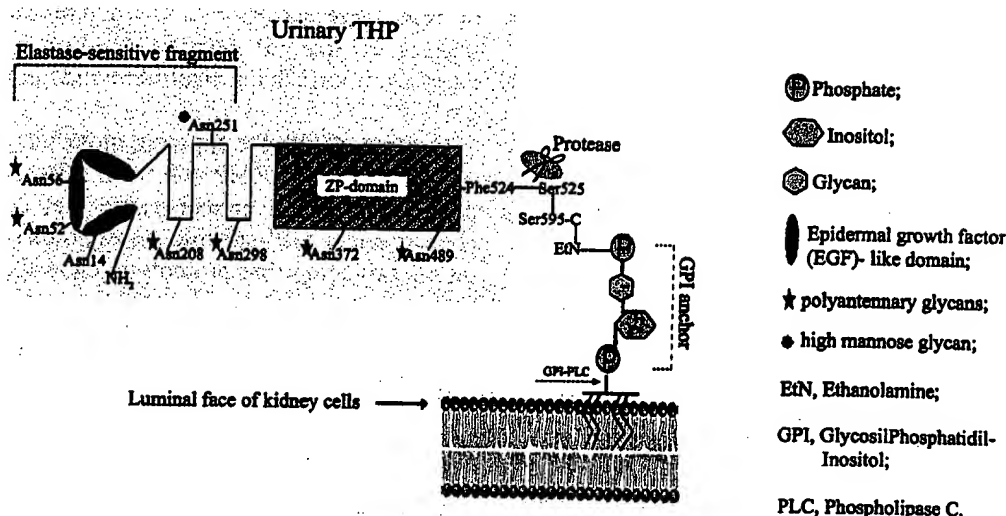


Fig 1. Structural model of urinary THP (yellow area) and its renal GPI-anchored counterpart.

These investigators isolated a glycoprotein from urine of pregnant women by a method that includes lectin-affinity chromatography. They named the protein uromodulin because in some conditions, it showed in vitro immunosuppressive activity. A similar property previously was shown to be present in THP.^{11,12} In 1987, the complementary DNA (cDNA) of uromodulin was cloned, and its identity with the cDNA of human THP was shown.^{13,14}

PHYSICAL-CHEMICAL STRUCTURE OF URINARY THP AND ITS RENAL GLYCOSYL PHOSPHATIDYLINOSITOL-ANCHORED COUNTERPART

The primary structure of human-THP has been predicted by sequencing the cDNA.^{13,14} On the

basis of the *N*-terminal sequence (Asp-Thr-Ser-Glu-Ala) found in urinary THP, Pennica et al¹³ assigned 616 amino acids to THP, in that the first 24 *N*-terminal amino acids predicted by cDNA represent the signal peptide lacking in the mature protein (we have used amino acid-mapping according to Pennica et al,¹³ to indicate both the *N*-glycosylation sites and all cited amino acids throughout the text). The protein includes 48 cysteine residues, 8 potential *N*-glycosylation sites, 3 epidermal growth factor domains, 1 zona pellucida (ZP)-like domain, and, at the C-terminus, 1 stretch of hydrophobic amino acids, similar to that of proteins that acquire a glycosyl phosphatidylinositol (GPI) anchor. This hydrophobic sequence acts as a signal for endoplasmic reticulum (ER)-transpeptidase, which, after cleav-

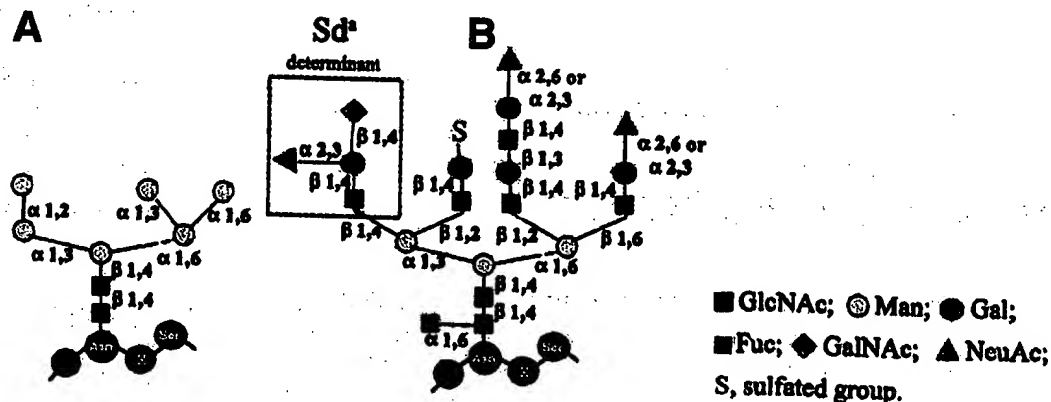


Fig 2. Representative structures of human THP *N*-glycans: (A) the major high-mannose sequence⁴⁷ and (B) the structure of a tetra-antennary chain showing the various nonreducing terminal units.^{38,39}

age of the hydrophobic peptide, adds the preformed GPI anchor to the "new" C-terminus (Fig 1).¹⁵ Rindler et al¹⁶ transfected THP cDNA into HeLa cells and showed that THP is a GPI-anchored protein. Fatty acids bound to the GPI anchor allow insertion of THP into the outer leaflet of the plasma membrane (Fig 1). DNA encoding THP in the rat, mouse, and calf also was sequenced,¹⁷⁻¹⁹ and the THP gene was reported present in all vertebrate classes.²⁰ The human THP gene was assigned to chromosome 16p12.3-16p13.11.²¹ The mouse THP gene promoter was isolated, and by using the transgenic mouse approach, a sequence of 3.0 kb was shown to be sufficient to drive kidney expression of a heterologous reporter gene.²²

On the basis of their amino acid sequence, a new class of GPI-anchored proteins, broadly homologous to THP, was identified. This class includes pancreatic glycoprotein 2 (GP2), which shows a 53% identity and 85% similarity to the human THP amino acid sequence^{17,23}; a sea urchin sperm membrane protein²⁴; and ZP2- and ZP3-glycoproteins, which participate in forming the transparent coat (zona pellucida) surrounding the eggs of all placental mammals.²⁵ GP2 is the major protein present in the zymogen secretory granule membrane of the pancreas and very likely is involved in the regulated release of zymogens.²⁶ Recently, Jovine et al²⁷ showed that (1) the peptide encompassing amino acids 1 to 291 of human THP is selectively degraded by pancreatic elastase, and (2) the residual C-terminal peptide chain of approximately 48 kd that THP shares with ZP2- and ZP3-glycoproteins (ZP domain) is responsible for polymerization of all these glycoproteins (Fig 1).

The first electron microscope and ultracentrifuge studies attributed to urinary THP an axial ratio of 250 and a molecular weight of 7×10^6 M_r .^{28,29} Subsequent studies showed that THP consists of protofilaments with a double-helix structure that, in polymer form, acquire a ribbon-shaped structure 1,500 to 4,000 nm in length and are organized in a large network.^{5,27} In SDS-PAGE, THP shows an apparent molecular weight of 80 kd in nonreducing conditions or 97 kd when the glycoprotein is reduced with 2-mercaptoethanol.^{7,30} The decrease in apparent molecular weight in nonreducing conditions depends on a high degree of polypeptide constraint imposed

by the large number of intrachain disulfide bridges, in that all cysteine residues are converted into intrachain disulfide bonds in urinary THP.³¹

Compared with the apparent molecular weight of human urinary THP, the renal GPI-anchored counterpart has a greater value that does not change under GPI-specific phospholipase treatment, indicating that proteolytic cleavage in the juxtamembrane region of the THP ectodomain is responsible for the urinary excretion that occurs.³⁰ Consistent with this, recombinant THP expressed by HeLa cells is released mainly devoid of ethanolamine, the residue responsible for binding the GPI anchor to the THP C-terminus³² (Fig 1). According to Fukuoka and Kobayashi,³³ proteolytic cleavage occurs between amino acids 524 (phenylalanine) and 525 (serine), ie, 66 amino acids upstream of the putative C-terminal residue bound to the GPI anchor.

THE GLYCOMOIETY STRUCTURE OF THP

Carbohydrates account for approximately 30% of the weight of human THP and consist mainly of *N*-linked glycans of di-, tri-, and tetra-antennary type^{6,34-36}; however, 1 *N*-glycosylation site carries high mannose sequences.³⁷ The tetra-antennary chains are elongated in a sizable percentage by repeating *N*-acetylglucosamine sequences and show marked heterogeneity because of differences in the extent of sialylation, fucosylation, and sulfation (Fig 2).^{38,39} Of the 8 potential *N*-glycosylation sites predicted by amino acid sequence, only 7 are actually glycosylated.⁴⁰

In addition, *N*-acetylgalactosamine (GalNAc) in β 1,4 linkage to galactose occurs in α 2,3 sialylated glycans, producing the GalNAc β 1,4(NeuAc α 2,3)Gal β 1,4GlcNAc-sequence known as Sd^a antigen (Fig 2).⁴¹ This antigen was originally described as a blood group determinant inherited as a dominant characteristic present in approximately 90% of the Caucasian population.⁴² The GalNAc residue is the immunodominant sugar, confirmed because THP from Sd^a-positive individuals contains 1% to 2% of GalNAc, whereas THP from Sd^a-negative individuals does not contain GalNAc.⁴³ Biosynthesis of Sd^a determinant depends on a specific β -GalNAc-transferase (Sd^a- β GalNAc-transferase) first described in our laboratory.^{44,45} Sd^a- β GalNAc-transferase activity is detected easily in the outer

medulla of human kidney, but not in the cortex, suggesting that the Sd^a antigen is carried prevalently by THP synthesized by cells from the thick ascending limb (TAL) of Henle's loop, rather than those from the early region of convoluted distal tubules.⁴⁶

In human THP, high-mannose glycans are carried by Asn₂₅₁,⁴⁰ and Man₆GlcNAc₂ is the preponderant structure over Man₇GlcNAc₂ and Man₅GlcNAc₂.^{37,47} Recombinant THP expressed by transfected HeLa cells also bears high-mannose sequences similar to those found in urinary THP, indicating that the occurrence of such a structure is host-cell independent, ie, it is imposed by the protein primary structure of THP.⁴⁸ The percentage of distribution of high-mannose sequences in calf THP is very similar to that of human THP,⁴⁹ whereas pig THP has a much greater molar percentage of Man₅GlcNAc₂ (47% of total high-mannose sequences in pig THP versus 8% in human THP; Cavallone et al, manuscript submitted). The difference in the branching chain of high-mannose glycans between pig and human THP might affect the interaction with type 1 fimbriated *Escherichia coli*.⁵⁰ Recently, O-linked chains also have been described as present in THP.⁵¹

A question that has given rise to much controversy is whether the carbohydrate moiety of THP from pregnant and nonpregnant women differs.^{49,51-54} An accurate recent analysis⁵⁵ established that THP from pregnant women (also termed uromodulin) shows a small increase in Man₇GlcNAc₂ molar percentage (Man₇GlcNAc₂ represents 35% of total high-mannose glycans in pregnant women versus 30% in nonpregnant women). Conversely, neither in the course of pregnancy nor 1 month after gestation was a significant change observed in the negative charge distribution of complex-type glycans, indicating no change in the content of sialic acid and sulfate residues.

LOCALIZATION AND BIOSYNTHESIS OF THP

When RNAs isolated from approximately 150 different cell tissues were hybridized using a large probe for human THP RNA, only RNA from human adult kidney gave a positive signal, indicating that this glycoprotein is exclusively produced by kidney cells.¹³ Immunofluorescence and immunochemical analyses by light and elec-

tron microscopy indicated that THP resides in kidney cells of TAL and early distal convoluted tubules.⁵⁶⁻⁶¹ When the cellular location of rat THP messenger RNA (mRNA) was investigated by in situ hybridization using a radiolabeled human THP complementary RNA antisense probe (>600 bp), a positive reaction was found along the entire length of the TAL, but not in the macula densa or distal convoluted tubules.⁶² This last observation is in agreement with previous immunofluorescence analyses of hamster and human kidney performed in the laboratory of Robin D. Marshall,^{59,60} indicating that macula densa cells lack THP. Gokhale et al⁶³ used an RNA probe specific for rat THP and observed a strongly positive mRNA signal in the TAL region alone, even in conditions in which rat THP was detectable in papillary tubules. This result strongly supports the idea that rat THP synthesis is restricted to the TAL of Henle's loop. In a Western blot, we detected a relative distribution of human THP in kidney lysates of the outer medulla approximately 4-fold greater than in the cortex.⁴⁶

The first ultrastructural studies by Hoyer et al⁵⁷ and Seiler and Hoyer⁵⁸ failed to show in rats that THP is distributed preferentially at the luminal face of TAL cells. Subsequently, Bachmann et al,⁶¹ using protein-gold immunocytochemistry, observed a prevalent localization of rat THP in vesicles in close spatial relationship to the Golgi cisternae, fused with the apical face of TAL cells, and visualized slight positive gold labeling on the basolateral face. Conversely, various experimental approaches support the notion that THP is delivered to the luminal plasma membrane: (1) the GPI anchor and N-linked glycans both act as signals for vectorial transport of glycoproteins to the apical surface of epithelial cells,^{64,65} (2) prevalent exposure of THP at the luminal cell surface is consistent with both substantial excretion in luminal fluid and scarcity in blood,⁶⁶ and (3) recombinant THP expressed by Madin Darby canine kidney (MDCK) cells is delivered entirely to the apical face and released in the corresponding medium.⁶⁷

THP has been found in kidney distal tubules of one 14-week human fetus and in fetal rats,⁶⁸ whereas that found in human amniotic fluid very likely is derived from the fetus.⁶⁹

The biosynthesis of recombinant THP ex-

pressed by HeLa cells shows that THP accumulates intracellularly as a precursor of approximately 86 kd, which is converted into the mature glycoprotein with an apparent molecular weight of 97 kd (in reducing conditions). This mass shift depends on the processing of most high-mannose glycans to polyantennary species.⁴⁸ Two bands with a difference in migration similar to that of precursor and mature forms of recombinant THP have been visualized by Western blotting in lysates from human and rat kidney.^{30,70} When HeLa cells expressing THP are treated with mannosamine, an inhibitor of GPI anchor biosynthesis, THP accumulates intracellularly and is significantly less exposed at the cell surface, indicating that GPI anchor addition is required for delivery to the plasma membrane.³² Figure 3 shows the routing of GPI-anchored THP to the cell surface.

Both the first radioimmunoassay⁸ and the latest enzyme-linked immunosorbent assay (ELISA) method⁷¹ have shown that on consecutive days, there is a large variation in daily THP excretion under physiological conditions. Various parameters, such as urine volume and excretion of calcium, sodium, potassium, and creatinine, have been evaluated in relationship to daily THP urinary release, as have physical exercise, body weight, and diet, but controversial results have been obtained.⁷¹⁻⁷⁴ Moreover, the half-life for THP turnover in a single individual was reported to vary from a minimum of 3 to a maximum of 168 hours.⁷⁴ Recently, a significant increment in renal expression of THP in rats given a high-salt diet was observed.⁷⁵ Because THP urinary excretion is a multistep process that includes biosynthesis rate, complex posttranslational processing, and proteolytic release from the GPI-anchored counterpart, it is not surprising to find a broad scatter in the final step.

PROPERTIES OF THP

Tendency to Gelation/Aggregation

One of the most peculiar features of THP in solution is its tendency to gelation/aggregation when sodium chloride concentration is close to 100 mmol/L or calcium chloride is 1 mmol/L.^{76,77} Both conditions usually occur in normal urine, and a method based on this property has been set up to purify THP. When urine of healthy individuals is filtered through a diatomaceous

earth filter, THP is entrapped selectively in the filter. Because gelation is reversed at a low ionic concentration, THP is desorbed from the filter by deionized water and isolated to homogeneity by means of 2-step filtration and washing.⁷⁸ Recently, the salt precipitation method originally described by Tamm and Horsfall was compared with the diatomaceous earth filter method for isolating THP from urine of proteinuric patients and pregnant women, and better purification was obtained using the latter method.⁷⁹

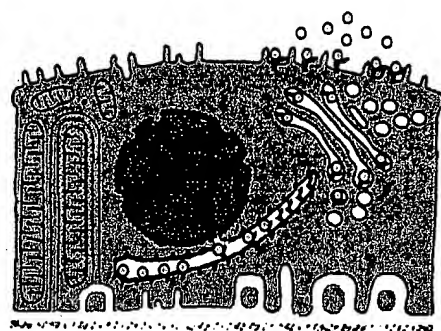
Interaction of THP With Other Proteins

Many reports indicate that THP binds to proteins present in urine, particularly those occurring in pathological conditions, but the tendency to gelation/aggregation of THP at a sodium chloride concentration close to 0.1 mol/L might have been responsible for overestimation of the binding property of THP. A clear interaction between immunoglobulin G (IgG) and urinary THP was observed by Rhodes et al.^{80,81} They used a Scatchard plot to analyze the interaction of a monomeric form of THP, obtained by urea solubilization, to avoid the salt-dependent insolubilization of polymeric THP. In their first article,⁸⁰ these researchers reported a very high-affinity association between THP and IgG with a dissociation constant (K_D) ranging from 10^{-13} to 10^{-10} mol/L. In the second report, the same investigators corrected the K_D value, which actually ranged from 10^{-9} to 10^{-7} mol/L, but confirmed the biological significance of the interaction.⁸¹

Differences in the binding of IgG to THP have been observed in patients with glomerulonephritis or interstitial nephritis and related to changes in the carbohydrate moiety of THP, whereas in children with lymphoid cell malignancies, the alteration in THP glycomoiety does not affect interaction with IgG.^{82,83}

IgG light chains also bind THP, and when they are largely present in urine, as in patients with myeloma, this interaction is responsible for the cast nephropathy accompanying this disease⁸⁴ (see Cast Nephropathy). Both C1q and C1 bind THP with an affinity that decreases significantly when the electrolyte concentration of the THP solution shifts from 0.020 to 0.15 mol/L of sodium chloride.^{85,86}

Owing to the heterogeneity of the large THP glycomoiety, there are many observations about



○ THP;
—● GPI anchor.

Fig 3. Schematic diagram of the biosynthesis and intracellular routing of GPI-anchored THP in a TAL cell. The addition of preformed GPI anchor to THP occurs in the ER: the membrane-bound form is transported to the Golgi complex, where glycans are fully processed, delivered to the luminal cell surface, and released in urine by a proteolytic cleavage (Fig 1). Note the large mitochondria and interdigitations in the basolateral membrane that are prominent in TAL cells.

its capacity for binding plant lectins, particularly those used as mitogens for lymphocyte blastogenesis, such as phytohemagglutinin (PHA), pokeweed mitogen, and concanavalin A.^{11,87} The strong inhibitory effect of urinary THP on PHA-induced lymphocyte blastogenesis is caused by the high-affinity interaction between PHA leucoagglutinin-subunits and tetra-antennary glycans, which are largely carried by THP.⁸⁸⁻⁹⁰ Very likely, the immunosuppressive activity assigned to THP from pregnant women (also termed uromodulin) depended on the competitive interaction of THP with PHA or pokeweed mitogen used in stimulating lymphocyte proliferation.^{10,14} Subsequent reports by the group of Muchmore^{52,91} showed that THP glycomoiety was entirely responsible for both in vitro inhibition of the antigen-specific lymphocyte proliferation and binding to recombinant interleukin-1 (IL-1) and recombinant tumor necrosis factor (TNF). According to Moonen and Williamson,⁹² soluble native cytokines do not bind to THP.

In our laboratory, we have shown that when high-mannose or complex-type THP glycopeptides are covalently bound to albumin, the neoglycoprotein inhibits the lymphocyte blastogenesis induced by 1-way mixed lymphocyte reaction. On this basis, we proposed that inhibition of lymphocyte proliferation is dependent on a multivalent interaction between THP glycans and

ligand(s) at the lymphocyte surface, and this interaction competes with the carbohydrate recognition system between effector and stimulator cells, which in the mixed lymphocyte reaction, mediates the blastogenesis.⁹³ Recently, it was reported that specific glycans of THP isolated from healthy adult males interact with IL-1 β , and glycans containing either *N*-acetylgalactosamine or sulfate residues in specific isomeric linkages are responsible for the inhibition of lymphocyte proliferation induced by tetanus toxoid.⁹⁴

Binding to and Activation of Leukocytes by THP

Several reports have shown that urinary THP binds to and activates leukocytes, including poly-

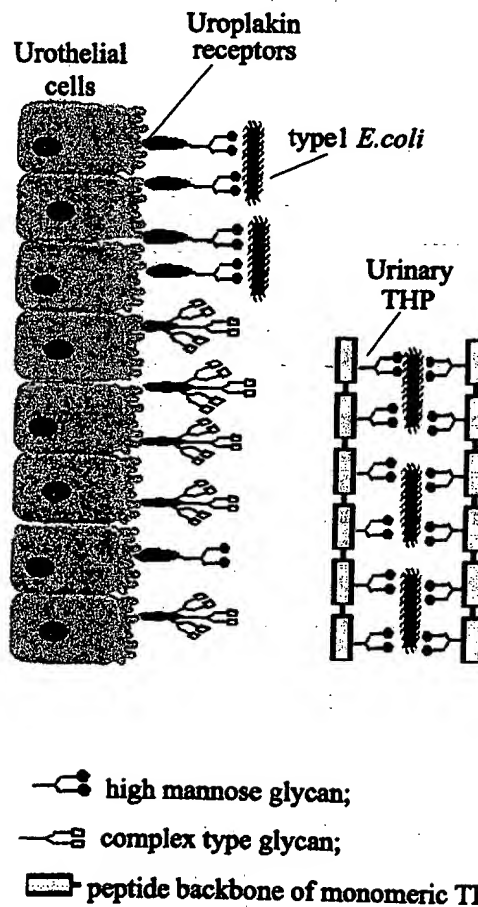


Fig 4. Binding of polymeric THP to type 1 *E. coli*, mediated by high-mannose glycans, competes with adhesion of pathogens to uroplakin receptors. Uroplakins are the major integral-membrane glycoproteins exposed at the luminal face of the bladder and urothelial tract. In the drawing of THP, polyantennary glycans have been omitted.

morphonuclear neutrophils (PMNs), lymphocytes, and monocytes. PMN activation is mediated by their interaction with THP (in monomeric form) through a single class of sialic acid-specific receptors exposed in great number at the surface of PMNs.^{95,96} Integrins also have been proposed to mediate the interaction between PMNs and THP through arginine-glycine-aspartic acid (RGD)-sequence mapping at position 142 to 144 of THP.⁹⁷ Recently, it was shown that recombinant THP produced by MDCK cells also binds to and activates PMNs.⁶⁷ According to Yu et al,⁹⁸ THP from pregnant women increases the phagocytic activity of PMNs and prostaglandin E₂ release, suggesting that a specific interaction occurs between THP and the membrane of PMNs. Similarly, blood mononuclear cells are activated by THP from pregnant women;⁹⁹ in this case, enhanced release of IL-1, IL-6, and TNF from monocytes very likely is responsible for the proliferation of both B and T lymphocytes. In a more recent study, Su et al¹⁰⁰ observed that THP from nonpregnant women also induces the secretion of TNF and expression of tissue factor by resting blood monocytes; however, both effects were several fold lower than those induced by THP from pregnant women (uromodulin).

Human monocytes isolated from peripheral blood and deprived of lymphocytes are able to phagocytize a particulate form of THP prepared from urine of healthy individuals and generate reactive oxygen metabolites, as well as release 95-kd gelatinase and other lysosomal enzymes.¹⁰¹ It is worth noting that the protection of mice against a lethal inoculum of *Listeria monocytogenes*, previously assigned to THP by Fontan et al,¹⁰² must be assigned to a minor protein (HGP92) also precipitated from normal urine by 0.58 mol/L of sodium chloride that shows an apparent molecular weight of 92 kd, very close to that of THP.¹⁰³

Binding of THP to Uropathogenic Strains of E coli

An interesting line of research concerns the role of THP in the kidney defense against urinary tract infections (UTIs), particularly those caused by *E coli*.¹⁰⁴⁻¹⁰⁷ UTIs occur in a large number of individuals (particularly women and children in developed countries), and the majority are caused by *E coli*.¹⁰⁸ Colonization is mediated by the

binding of lectin-like adhesins present on *E coli* fimbriae to carbohydrate structures carried by glycoproteins and glycolipids exposed at the cell surface. *E coli* fimbriae are classified according to their sugar specificity: type 1, type P, and type S recognize the high-mannose glycan, Gal α 1,4Gal β -sequence present in globoseries of glycolipids and the NeuA α 2,3Gal sequence of sialylated glycans, respectively.¹⁰⁹ Urinary THP carries both high-mannose and NeuA α 2,3Gal sequences and thus may be considered a ligand for both type 1 and type S fimbriated *E coli*. There is evidence that type 1 *E coli* strains represent the predominant phenotypic variants of isolates from patients with UTIs,¹¹⁰ and uroplakin Ia and Ib (the most abundant integral membrane glycoproteins of the luminal surface in urothelial cells) behave as efficient cell receptors for type 1 fimbriated *E coli*.¹¹¹⁻¹¹⁴ Pak et al¹⁰⁷ recently showed that THP binds type 1 fimbriated *E coli* in vitro and efficiently competes with uroplakin Ia and Ib in binding to these pathogens. These results support the notion that in vivo, urinary THP represents a protective agent against UTI. Figure 4 shows the putative role of urinary THP in protection against UTIs.

CLINICAL RELEVANCE OF URINARY THP

Urinary THP is believed to be involved in the pathogenesis of various disorders of distal nephrons and the urinary tract, such as cast nephropathy, urolithiasis, and tubulointerstitial nephritis (TIN). Moreover, reduced urinary THP excretion is considered a reliable index of distal tubular cell damage. Finally, a very recent study showed that familial juvenile hyperuricemic nephropathy (FJHN) and autosomal dominant medullary cystic kidney disease 2 (MCKD2) arise from mutations of the THP gene.¹¹⁵

Cast Nephropathy

Microscopic observation of casts allows one to distinguish casts according to size and morphological characteristics. Although size reflects the shape of the tubules in which casts are formed, eg, they appear convoluted when formed in the convolute part of a distal nephron, a classification based on morphological characteristics consists of hyaline, granular, fatty, and leukocyte casts. THP is by far the predominant protein of hyaline casts, but also is present in the matrix of

casts in which plasma proteins or hemoglobin are abundant, as well as in granular casts containing debris from damaged kidney cells or blood cells.¹¹⁶

McQueen¹¹⁷ first proposed that THP was present in urinary casts. Subsequently, using a fluorescence antibody technique, he presented direct evidence of the widescale occurrence of THP in the matrix of urinary casts.¹¹⁸ Interestingly, in his first article, McQueen¹¹⁷ also reported that myeloma protein precipitates THP in aqueous solution. A few years later, Fletcher et al¹¹⁹ showed that THP isolated from casts of 4 patients with nephrotic syndrome and urine of healthy individuals showed a very similar amino-acid composition and carbohydrate content. More recently, Fairley et al¹²⁰ examined the protein composition of urinary casts from 60 patients with glomerulonephritis and 46 healthy individuals subjected to strenuous exercise. In addition to THP, plasma proteins, particularly IgA, IgG, or IgM, were found in the cast matrix of all patients, but THP was the only protein detected in hyaline casts from 41 healthy individuals, whereas in the remaining 5 individuals, fibrin, C3, and C1q, but not immunoglobulins, were present.

Recently, Fogazzi and Testanera¹²¹ reported that on the basis of histological examinations and chemical analyses of kidney and urinary casts, Carlo R. Rovida, who ran the Institute of Clinical Medicine at the University of Turin (Italy) from 1874 to 1877, first described a specific protein, termed *cilindrina*, produced by kidney tubular cells as the major constituent of hyaline casts.

The involvement of THP in hyaline cast formation is facilitated by its tendency to gelation/aggregation at a salt concentration close to isosmolarity.⁷⁶ It is worth noting that in the TAL, the tubular fluid in physiological conditions is at a low electrolyte concentration in that epithelial cells are not permeable to water, whereas chloride, sodium, and potassium are actively absorbed through Na,K-adenosine triphosphatase activity. Thus, in physiological tubular fluid in which THP is released from the GPI-anchored counterpart, the electrolyte concentration disfavors the gelation/aggregation of THP. There is evidence that removal of all *N*-linked glycans by *N*-glycanase treatment negatively affects the gel-forming tendency of THP.^{122,123}

The presence in urine of plasma proteins,

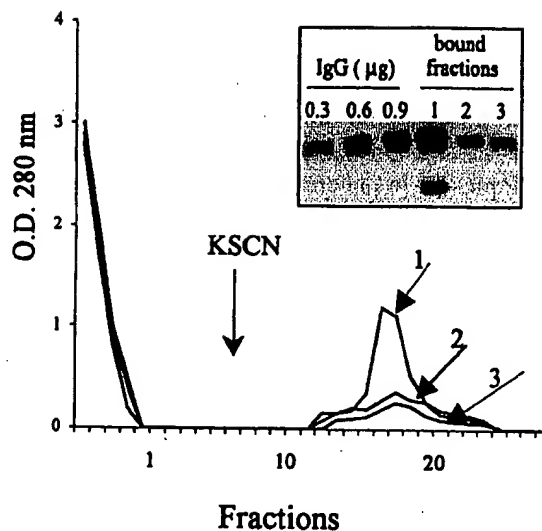


Fig 5. Representative profiles of affinity chromatography on a THP-CNBr-Sepharose column of urine from a patient with nephrotic syndrome (proteinuria, 3.7 g/L of protein). Arrows 1, 2, and 3 indicate the chromatography profile of urine dialyzed against 0.02, 0.08, and 0.15 mol/L of sodium chloride, respectively. (Insert) Western blot of pooled fractions containing bound proteins eluted by 3 mol/L of KSCN; lanes 1, 2, and 3 correspond to the 1, 2, and 3 chromatography. SDS-PAGE was performed in nonreducing conditions, and the blot was developed using anti-IgG antibodies.

particularly IgG and IgG light chains, is a crucial condition for cast formation. We observed that when IgG is excreted heavily by proteinuric patients, it binds to THP covalently linked to Sepharose in an amount that decreases significantly when sodium chloride concentration is increased from 0.02 to 0.15 mol/L. Figure 5 shows results of urine analysis from a patient with nephrotic syndrome (proteinuria, 3.7 g/L of protein). Similar results were obtained when proteinuric patients with other nephropathies were analyzed. These results support the notion that the electrolyte concentration of tubular fluid has a part in cast formation, particularly when IgG is present in urine.

Many studies^{84,124-127} reported the interaction between IgG light chains and THP. The THP sequence encompassing amino acids 225 to 233 appears to be crucial for binding to IgG light chains,¹²⁶ and the third complementary-determining region of both κ and λ light chains is required for binding to THP.¹²⁷ Leboulleux et al,¹²⁸ using an ELISA procedure, found that 5 of 12 samples of light chains from patients with cast nephropa-

thy did not react with THP and suggested that both a low affinity of certain light chains and differences in cast pathogenesis may explain these results. Consistent with the implication of THP in cast formation, GP2, a homologue of THP present in pancreas cells, is the major component of intraductal plugs of pancreatic juice.¹²⁹

Urolithiasis (Stone Disease)

Although the water-conservation function of the kidney produces supersaturation of various insoluble salts, such as calcium oxalate, calcium phosphate, and urate salts, only approximately 3% of the adult population of the Western hemisphere has nephrolithiasis.¹³⁰ The relatively low incidence of this disease depends essentially on 2 conditions: the presence in tubular fluid and urine of stone-formation inhibitors and the complex process required for the growth of microscopic crystal to a size producing stone disease. Stone formation usually takes place in various phases: the first consists of nucleation of supersaturated salts. Nucleation also may occur on heterogeneous surfaces present in tubular fluid or urine, eg, cell debris or urinary casts; subsequently, the microscopic nuclei undergo aggregation and grow into macroscopic stones.¹³⁰ Adherence of crystal aggregates to the tubular cell surface also is considered to have an important role in stone formation.¹³¹⁻¹³³

THP was found in human kidney stones in 1965 by Keutel,¹³⁴ and in 1973, Grant et al,¹³⁵ using a radioimmunoassay method, showed that THP content ranges from 0.002 to 5.07 mg/g of renal and bladder calculi, and there is no correlation between amount of THP and qualitative composition of inorganic components of stones.

The involvement of urinary THP in crystal nucleation has not been consistently proved,¹³⁶ whereas in an *in vivo* rat model, THP does not appear to mediate the initiation of crystal formation.¹³⁷ The inhibitory effect of THP in crystal aggregation and growth has been described in the case of both calcium oxalate¹³⁸⁻¹⁴⁰ and hydroxyapatite stones.¹⁴¹ There is consensus that inhibition of crystal growth in normal urine is caused mainly by urinary macromolecules, rather than low-molecular-weight components, and this property has been associated with the polyanionic structure of the major inhibitors.¹⁴² For instance, heparin and chondroitin sulfate are rich

in SO_4^{2-} groups,¹⁴³ whereas nephrocalcin, osteopontin (uropontin), and F1 activation peptide of prothrombin belong to the aspartic acid-rich protein superfamily.¹⁴⁴⁻¹⁴⁶ THP also is a polyanionic macromolecule (3.5 isoelectric point) because of the large extent of sialylation (18 residues/molecule according to Dustan et al⁹) and the presence of sulfate groups bound to *N*-linked glycans.³⁸ Recently, the presence in the luminal face of tubular cells of sialylated glycans carried by glycoproteins and glycolipids has been seen as crucial in the nucleation of calcium oxalate dihydrate crystals.¹⁴⁷ Conversely, adhesion of uric acid crystals to renal cells in culture (MDCK and BSC-1 line) was suggested to be mediated mainly by hydrogen bonds and hydrophobic interactions.¹⁴⁸ Because GPI-anchored THP is one of the main sialylated glycoproteins exposed at the luminal face of the first tract of distal nephrons, it should be involved in the calcium oxalate nephrolithiasis process even before it is released in urinary fluid. A recent study¹⁴⁹ reported decreased THP expression in the kidney of rats treated for 8 weeks with ethylene glycol to obtain calcium oxalate urolithiasis. However, reduced expression was detected only after 4 weeks of treatment, when crystal aggregates of calcium oxalate were visualized in the kidney of treated rats.

The difference in degree of sialylation between urinary THP from healthy individuals and recurrent calcium oxalate renal stone formers has been investigated extensively. Some studies¹⁵⁰⁻¹⁵³ reported an increase in isoelectric point of THP from recurrent calcium oxalate stone formers, probably dependent on a reduced sialic acid content. However, this observation was not confirmed subsequently.^{63,154} Despite this, partial removal of sialic acid from THP by neuraminidase treatment results in loss of *in vitro* inhibition of urinary crystal aggregation,^{155,156} consistent with the notion that polyanionic structure is crucial for inhibition of calcium oxalate crystal aggregation.

The involvement of urinary macromolecules in the modulation of crystal aggregation, as well as their participation in stone formation, was analyzed in some studies by using diluted urine or urine processed by centrifugation or filtration. The last 2 procedures, particularly filtration, significantly reduced THP content in processed

urine.¹⁵⁷ It is evident that this behavior may have been responsible for discrepancies in results concerning the role of THP as a modulator of urolithiasis, particularly when this role was compared with that of other urinary modulators that do not significantly change concentration on urine processing.

TIN

Bacterial infections, vesicoureteral reflux, exposure to heavy metal, and antibiotic or analgesic intake are the major etiologic agents of TIN, which is characterized in the first phase by infiltration of inflammatory cells (mononuclear cells, lymphocytes, plasma cells, and PMNs) into kidney interstitium and subsequently by interstitial fibrosis and tubular atrophy.¹⁵⁸ Clinical studies indicated that renal diseases, namely, chronic interstitial nephritis, medullary cystic disease, reflux nephropathy, and rejecting renal allografts, very often are accompanied by abnormal THP deposits in the tubular interstitium, and there is an immunologic response to THP.¹⁵⁹⁻¹⁶³ THP is a powerful autoantigen, and immune deposits containing THP and anti-THP antibodies have been localized in the intercellular space of TAL in rats, mice, and rabbits challenged with homologous THP.^{58,164-167} In all likelihood, healthy mammals, including humans, do not produce anti-THP antibodies because the exclusive localization of THP at the luminal face of tubular cells segregates the protein from the immune system. Segregation may be abolished by various alterations to cells expressing THP, such as loss of their apical/basolateral polarity and the consequent release of THP from the wrong side, as well as conditions altering cell integrity. Non-vectorial delivery of THP to the luminal surface also may occur in conditions that alter the routing of THP along the exocytic pathway. Malagolini et al³² observed that under treatment with monensin, an ionophore that interferes with the function of the Golgi apparatus, a THP that is not fully glycosylated and not exposed at the cell surface is released from cells expressing THP. Because the GPI anchor is a sorting signal to the cell apical face,⁶⁴ if the GPI anchor is lost along the exocytic pathway, THP also might be released from the basolateral face and enter the peritubular capillaries; tubular basement mem-

branes are highly permeable to macromolecules.¹⁶⁸

Several studies have shown that interstitial deposits of THP and THP-immune complexes frequently are surrounded by neutrophils, mononuclear cells, and plasma cells.¹⁵⁹⁻¹⁶⁴ Although binding of THP to neutrophils may be responsible for the acute inflammatory response,⁹⁵⁻⁹⁷ binding to mononuclear cells may extend the reaction to the chronic phase characterized by fibrosis.^{101,165} Although the proinflammatory activity of THP has been put forward as involved in TIN pathogenesis,^{96,101} both clinical and experimental observations indicate that abnormal THP deposits in the kidney do not have a crucial role in inflammatory processes. For instance, on the basis of 124 renal biopsies of patients with various nephropathies, Chambers et al¹⁶⁹ concluded there is no correlation between THP accumulation in the interstitium and tubulointerstitial damage, although in the majority of patients examined, THP antibodies were present. The absent or low inflammatory response to extratubular THP deposits (particularly PMN infiltration) also was reported in renal allografts and patients with urinary extravasation.¹⁷⁰⁻¹⁷² Again, in experimental models of unilateral ureteral obstruction in rats or mice, as used by Dziukas et al¹⁷³ and Fasth et al,¹⁷⁴ THP accumulation in the interstitium is not accompanied by inflammatory response. These investigators suggested that in urine extravasation, toxin components other than THP may be responsible for the inflammation and TIN pathogenesis.

When transfected HeLa cells stably expressing cell surface-anchored THP were incubated with human neutrophils, no binding was found, but opsonization of cells with anti-THP antibodies resulted in (1) dramatic adhesion and myeloperoxidase activation of PMNs, (2) a significant increase in THP release, and (3) exit of the partially processed THP from the cells. These results support the notion that after the autoimmune response, cell surface-anchored THP also may contribute to the pathogenesis of TIN.¹⁷⁵

Involvement of THP in tubular cell damage also has been related to its ability to bind to C1q, the component of the complement pathway responsible for initiating the complement cascade.^{85,86} There is evidence that in some renal diseases (eg, nephrotic syndrome), complement

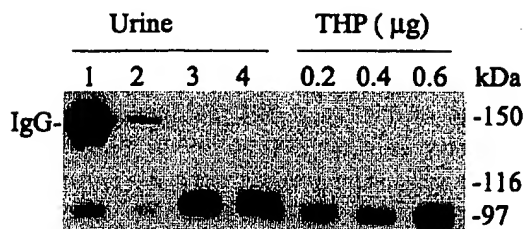


Fig 6. Western blot of urine samples from patients with lupus nephritis (lane 1) and MCKD (lane 2) and 2 healthy individuals (lanes 3 and 4). An identical percentage of daily urinary volume (0.5%) was applied in each lane. Urinary THP used as a marker was isolated from pooled urine samples of healthy individuals. SDS-PAGE was performed in nonreducing conditions, and the blot was developed using both anti-THP antibodies and anti-IgG antibodies. As expected, the latter antibody visualized a large presence and a trace of IgG in urine from patients with lupus nephritis and MCKD, respectively, and absence of IgG in healthy individuals.

factors are excreted copiously and activated in urine.¹⁷⁶ Thus, either complement per se or the THP-C1q complex may accumulate in the interstitium and have an important role in TIN pathogenesis.

THP Excretion in Nephropathies

Abnormalities in daily urinary excretion of THP have been detected in several pathological states of the kidney and urinary tract, and decreased excretion is considered a reliable index of damage to the TAL cells that synthesize THP.^{73,177,178} Our laboratory detected a marked reduction in daily THP excretion in a patient with medullary polycystic kidney disease (Fig 6). In active lupus nephritis, decreased THP excretion was related to impairment of the distal nephron function that accompanies the active phase of the disease¹⁷⁹ (Fig 6). Again, marked reduction in urine THP from infants with hyperprostaglandin E syndrome was related to a congenital defect of the distal nephron.¹⁸⁰ THP excretion also is reduced in urine of stone formers, very likely because stones per se damage the tubular epithelium of the TAL.¹⁸¹⁻¹⁸³ Interestingly, a study¹⁸⁴ of THP excretion after uninephrectomy in kidney donors has shown a persistent increase (~40%) in THP excretion from the kidney that remains in the donor and good correlation between THP excretion rate and glomerular filtration rate.

Urinary THP has been studied in diabetes mellitus, particularly the insulin-dependent

type.¹⁸⁵⁻¹⁹³ A reduction in THP excretion was observed consistently when diabetic nephropathy is characterized by a reduction in renal mass, which usually occurs at a late stage of disease.¹⁸⁵⁻¹⁹⁰ Conversely, other investigators observed increased excretion in the early stage of the disease, as well as during acute euglycemia and water load.¹⁹¹⁻¹⁹³ In experimental diabetes induced in rats by streptozotocin, THP excretion was increased markedly, an effect supposed to be associated with distal tubular dysfunction.^{194,195} According to Agardh et al,¹⁹⁵ greater excretion is attenuated by compounds that inhibit both the production of advanced glycation end products and oxidative stress, 2 processes involved in the development of diabetic nephropathy.

Mutations to the THP Gene in FJHN and MCKD2

The autosomal dominant MCKD2 and FJHN, very likely 2 facets of the same disease, are characterized by hyperuricemia, medullary cysts, interstitial nephritis, and progressive renal failure.¹⁹⁶ By genome-wide linkage mapping in Italian, Czech, and Belgian families, new loci for MCKD2 and FJHN were identified in the regions of chromosome 16p11.2 and 16p12,¹⁹⁶⁻¹⁹⁸ close to the localization of the human THP gene.²¹ Mutations of the THP gene were identified very recently by Hart et al¹¹⁵ in several individuals from 3 unrelated families with FJHN and 1 family with MCKD2. The missense mutations identified consist of the deletion or addition of 1 cysteine residue and 1 frame deletion of 9 amino acids. These results provide the first demonstration that FJHN and MCKD2 arise from allelic mutations of the THP gene and suggest that an alteration to the tertiary structure of THP, particularly the misfolding caused by the uncoupling of at least 1 Cys-Cys bond, is responsible for the disease(s).

We observed³² that when HeLa cells expressing THP are treated with an exogenous reducing agent, such as 2-mercaptoethanol, routing along the exocytic pathway of partially reduced THP is delayed considerably, indicating that completion of intrachain disulfide bonds is required for a regular exit from the ER and delivery of THP to the cell surface. On this basis, one may postulate that misfolded THP produced in MCKD2 and FJHN undergoes a similar fate, and accumula-

tion in the ER may result in altered biosynthesis, eg, impairment of the GPI addition and glycosylation processing, 2 events crucial for the delivery of THP to the luminal surface of the tubular epithelium. These effects may explain the TIN associated with the disease(s).

THP IN BIOTECHNOLOGY

Observations that the THP gene is transcribed exclusively by TAL cells and THP is largely excreted in urine recently have been used for the transgenic production of human therapeutic proteins.^{199,200} This approach is based on recently developed technologies that allow one to generate transgenic mice by using a mouse gene promoter to direct the expression of proteins selectively released in urine.²⁰¹ In comparison to the expression of human therapeutic proteins in milk of transgenic livestock, production by kidney cells and the consequent release in urine appears to offer a much more cost-effective advantage in that urine contains few proteins or lipids; a condition that facilitates isolation of the recombinant protein to homogeneity. In this way, recombinant human α_1 -antitrypsin was isolated from transgenic mice. It shows a broad similarity in its activity to that produced by human hepatocytes.¹⁹⁹ When the same approach was used to produce human recombinant erythropoietin, its expression and excretion in transgenic mice was accompanied by disease symptoms similar to polycythemia in humans, confirming that the THP promoter is effective in directing the production of proteins capable of exerting an *in vivo* effect.²⁰⁰

Recently, research in the field of genomic manipulation acquired a new strategy, referred to as the Cre/loxP site-directed recombinant system.²⁰² This strategy provides an advantageous tool for spatially and temporally modulated somatic mutations. When stable transgenic mice expressing Cre recombinase, under a tissue-specific promoter, are mated with mice harboring the loxP DNA sequence, a new mouse line may be obtained in which the gene disruption mediated by Cre recombinase occurs only in cells in which the promoter is active.²⁰² Striklett et al²⁰³ used this strategy and, using a THP promoter, found that Cre-recombinase is specifically expressed and active in cells of TAL. As these investigators emphasized, this approach intro-

duces a new tool in specifically exploring the function of the TAL, which represents one of several regions of the kidney that variously contribute to renal physiological states.

CONCLUSION

Although the bulk of evidence shows the involvement of THP in pathological states of the kidney and urinary tract, there are no clear indications about its physiological role, although the structural, genetic, and cytological characterization largely has been clarified in the last 20 years. In the late 1980s, great enthusiasm accompanied the observation that urinary THP/uromodulin could have an immunoregulatory function in that it inhibits *in vitro* lymphocyte blastogenesis and interacts with cytokines.²⁰⁴ Some considerations may be proposed to explain why this line of research has not confirmed initial expectations and hopes for the therapeutic use of THP. First, results obtained *in vitro* have not been carefully evaluated with respect to their *in vivo* relevance; in physiological conditions, THP is expressed exclusively by TAL cells, delivered to the luminal surface, and excreted in urinary fluid. Thus, it is improbable that a protein present in a cell compartment and fluid, where very few cells mediating the immune response occur, may show an immunoregulatory function.

The second consideration concerns the demonstration that the glycomoiety of THP is entirely responsible for inhibiting lymphocyte proliferation and cytokine binding.^{52,91} Because completion of the carbohydrate moiety of a glycoprotein is dictated specifically by enzymes (glycosidases and glycosyltransferases) from cells responsible for expressing it,²⁰⁵ large-scale production of a functionally active THP cannot be made by the usual cost-effective biotechnological techniques. Last, but not least, the property by which THP behaves as an efficient autoantibody prevents it from being used *in vivo*.

Considering that release of THP in the urinary fluid from the cell-surface GPI-anchored counterpart is relatively constant and abundant in physiological conditions and all mammals excrete THP, one may assume that its presence in urine is an advantage very likely imposed by selective pressure. Thus, the question is, what condition(s) served as a selective agent for this process? One possible answer is that urinary THP affords effi-

cient protection toward the most frequent infections of the urinary tract by binding uropathogenic strains of *E. coli*.¹⁰⁷ The luminal cell surface of the respiratory and intestinal epithelium is covered by mucus, an effective agent in preventing adhesion of pathogens to glycoproteins and glycolipids exposed at the luminal plasma membrane. This protection is absent from the urinary tract in that no mucus (or only a very small amount) covers the luminal cells, particularly in the bladder, in which urine constantly resides. Because infections are caused mainly by *E. coli* strains that enter the urinary tract by an ascending route from intestinal flora,²⁰⁶ a real advantage seems to be offered by the presence of THP as a soluble ligand because, following the route and fate of urine, it may interact with the pathogens any time after their entry into the urinary tract and eventually be eliminated from the body. The occurrence of THP in urine as a large homopolymer also is a very advantageous property in that it behaves as a multivalent ligand, ie, it binds pathogens with a high affinity. In this context, addition of the GPI anchor also may be an advantage from an evolutionary point of view because the GPI anchor is a selective signal for delivering glycoproteins to the luminal cell surface of tubular epithelium and for the restricted release of THP into the urinary fluid.

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REFERENCES

1. Tamm I, Horsfall FL: Characterization and separation of an inhibitor of viral hemagglutination present in urine. *Proc Soc Exp Biol Med* 74:108-114, 1950
2. Tamm I, Horsfall FL: A mucoprotein derived from human urine which reacts with influenza, mumps, and Newcastle disease viruses. *J Exp Med* 95:71-97, 1952
3. Gottschalk A: Carbohydrate residue of a urine mucoprotein inhibiting influenza virus haemagglutination. *Nature* 170:662-663, 1952
4. Odin L: Carbohydrate residue of a urine mucoprotein inhibiting influenza virus haemagglutination. *Nature* 170:663-664, 1952
5. Bayer ME: An electron microscope examination of urinary mucoprotein and its interaction with influenza virus. *J Cell Biol* 21:265-274, 1964
6. Fletcher AP, Neuberger A, Ratcliffe WA: Tamm-Horsfall urinary glycoprotein. The chemical composition. *Biochem J* 120:417-424, 1970
7. Fletcher AP, Neuberger A, Ratcliffe WA: Tamm-Horsfall urinary glycoprotein. The subunit structure. *Biochem J* 120:425-432, 1970
8. Grant AM, Neuberger A: The development of a radioimmunoassay for the measurement of urinary Tamm-Horsfall glycoprotein in the presence of sodium dodecyl sulphate. *Clin Sci* 44:163-179, 1973
9. Dunstan DR, Grant AM, Marshall RD, Neuberger A: A protein immunologically similar to Tamm-Horsfall glycoprotein, produced by cultured baby hamster kidney cells. *Proc Soc Lond (B Biol Sci)* 186:297-316, 1974
10. Muchmore AV, Decker JM: Uromodulin: A unique 85-kilodalton immunosuppressive glycoprotein isolated from urine of pregnant women. *Science* 229:479-481, 1985
11. Serafini-Cessi F, Franceschi C, Sperti S: Specific interaction of human Tamm-Horsfall glycoprotein with leucoagglutinin, a lectin from *Phaseolus vulgaris* (red kidney bean). *Biochem J* 183:381-388, 1979
12. Franceschi C, Licastro F, Chiricolo M, Serafini-Cessi F, Tabacchi P: Monosaccharides and Tamm-Horsfall glycopeptide inhibit allogenic antigen-induced lymphocyte blastogenesis in one-way mixed lymphocyte reaction, in Bog-Hansen TC (ed): *Lectins—Biology, Biochemistry, Clinical Biochemistry*, vol II. Berlin, Germany, de Gruyter, 1982, pp 153-164
13. Pennica D, Kohr WJ, Kuang WJ, et al: Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein. *Science* 236:83-88, 1987
14. Hession C, Decker JM, Sherblom AP, et al: Uromodulin (Tamm-Horsfall glycoprotein): A renal ligand for lymphokines. *Science* 237:1479-1484, 1987
15. Ferguson MA, Williams AF: Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. *Annu Rev Biochem* 57:285-320, 1988
16. Rindler MJ, Naik SS, Li N, Hoops TC, Peraldi MN: Uromodulin (Tamm-Horsfall glycoprotein/uromucoid) is a phosphatidylinositol-linked membrane protein. *J Biol Chem* 265:20784-20789, 1990
17. Fukuoka S, Freedman SD, Yu H, Sukhatme VP, Scheele GA: GP-2/THP gene family encodes self-binding glycosylphosphatidylinositol-anchored proteins in apical secretory compartments of pancreas and kidney. *Proc Natl Acad Sci U S A* 89:1189-1193, 1992
18. Prasad K, Bates J, Badgett A, et al: Nucleotide sequence and peptide motifs of mouse uromodulin (Tamm-Horsfall protein): The most abundant protein in mammalian urine. *Biochim Biophys Acta* 1260:328-332, 1995
19. Yu H, Papa F, Sukhatme VP: Bovine and rodent Tamm-Horsfall protein (THP) genes: Cloning, structural analysis, and promoter identification. *Gene Expr* 4:63-75, 1994
20. Badgett A, Kumar S: Phylogeny of Tamm-Horsfall protein. *Urol Int* 61:72-75, 1998
21. Pook MA, Jeremiah S, Scheinman SJ, Povey S, Thakker RV: Localization of the Tamm-Horsfall glycoprotein (uromodulin) gene to chromosome 16p12.3-16p13.11. *Ann Hum Genet* 57:285-290, 1993
22. Zhu X, Cheng J, Gao J, et al: Isolation of mouse THP gene promoter and demonstration of its kidney-specific activity in transgenic mice. *Am J Physiol (Ren Physiol)* 282:608-617, 2002

23. Hoops TC, Rindler MJ: Isolation of the cDNA encoding glycoprotein-2 (GP-2), the major zymogen granule membrane protein. Homology to uromodulin/Tamm-Horsfall protein. *J Biol Chem* 266:4257-4263, 1991
24. Mendoza LM, Nishioka D, Vacquier VD: A GPI-anchored sea urchin sperm membrane protein containing EGF domains is related to human uromodulin. *J Cell Biol* 121:1291-1297, 1993
25. Wassarman PM: Mammalian fertilization: Molecular aspects of gamete adhesion, exocytosis, and fusion. *Cell* 96:175-183, 1999
26. Rindler MJ, Hoops TC: The pancreatic membrane protein GP-2 localizes specifically to secretory granules and is shed into the pancreatic juice as a protein aggregate. *Eur J Cell Biol* 53:154-163, 1990
27. Jovine L, Qi H, Williams Z, Litscher E, Wassarman PM: The ZP domain is a conserved module for polymerization of extracellular proteins. *Nat Cell Biol* 4:457-461, 2002
28. Porter KR, Tamm I: Direct visualization of a mucoprotein component of urine. *J Biol Chem* 212:135-141, 1955
29. Maxfield M: Molecular forms of human urinary mucoprotein present under physiological conditions. *Biochim Biophys Acta* 49:548-559, 1961
30. Cavallone D, Malagolini N, Serafini-Cessi F: Mechanism of release of urinary Tamm-Horsfall glycoprotein from the kidney GPI-anchored counterpart. *Biochem Biophys Res Commun* 280:110-114, 2001
31. Hamlin LM, Fish WW: Physical properties of Tamm-Horsfall glycoprotein and its glycopolyptide. *Int J Pept Protein Res* 10:270-276, 1977
32. Malagolini N, Cavallone D, Serafini-Cessi F: Intracellular transport, cell-surface exposure and release of recombinant Tamm-Horsfall glycoprotein. *Kidney Int* 52:1340-1350, 1997
33. Fukuoka SI, Kobayashi KI: Analysis of the C-terminal structure of urinary Tamm-Horsfall protein reveals that the release of glycosyl phosphatidylinositol-anchored counterpart from the kidney occurs by phenylalanine-specific proteolysis. *Biochem Biophys Res Commun* 289:1044-1048, 2001
34. Afonso AMM, Charlwood PA, Marshall RD: Isolation and characterization of glycopeptides from digests of human Tamm-Horsfall glycoprotein. *Carbohydr Res* 89:303-319, 1981
35. Serafini-Cessi F, Malagolini N, Dall'Olio F: A tetraantennary glycopeptide from human Tamm-Horsfall glycoprotein inhibits agglutination of desialylated erythrocytes induced by leucoagglutinin. *Biosci Rep* 4:973-978, 1984
36. Hard K, Van Zadelhoof G, Moonen P, Kamerling JP, Vliegthart JFG: The Asn-linked carbohydrate chains of human Tamm-Horsfall glycoprotein of one male. *Eur J Biochem* 209:895-915, 1992
37. Serafini-Cessi F, Dall'Olio F, Malagolini N: High-mannose oligosaccharides from human Tamm-Horsfall glycoprotein. *Biosci Rep* 4:269-274, 1984
38. van Rooijen JJM, Kamerling JP, Vliegthart JFG: Sulfated di-, tri- and tetraantennary *N*-glycans in human Tamm-Horsfall glycoprotein. *Eur J Biochem* 256:471-487, 1998
39. van Rooijen JJM, Kamerling JP, Vliegthart JFG: The abundance of additional *N*-acetylglucosamine units in *N*-linked tetraantennary oligosaccharides of human Tamm-Horsfall glycoprotein is a donor-specific feature. *Glycobiology* 8:1065-1075, 1998
40. van Rooijen JJM, Voskamp AF, Kamerling JP, Vliegthart JFG: Glycosylation sites and site-specific glycosylation in human Tamm-Horsfall glycoprotein. *Glycobiology* 9:21-30, 1999
41. Donald ASR, Yates AD, Soh CPC, Morgan WTJ, Watkins WM: A blood group Sd^a-active pentasaccharide isolated from Tamm-Horsfall urinary glycoprotein. *Biochem Biophys Res Commun* 115:625-631, 1983
42. Serafini-Cessi F: The Sd^a antigen and its biosynthetic enzyme: Differentiation-dependent and onco-developmentally regulated expression. *Trends Glycosci Glycotechnol* 42:279-295, 1996
43. Soh CP, Morgan WTJ, Watkins WM, Donald ASR: The relationship between the *N*-acetylglucosamine content and the blood group Sd^a activity of Tamm and Horsfall urinary glycoprotein. *Biochem Biophys Res Commun* 93:1132-1139, 1980
44. Serafini-Cessi F, Dall'Olio F: Guinea-pig kidney β -*N*-acetylglucosaminyltransferase towards Tamm-Horsfall glycoprotein. Requirement of sialic acid in the acceptor for transferase activity. *Biochem J* 215:483-489, 1983
45. Serafini-Cessi F, Dall'Olio F, Malagolini N: Characterization of *N*-acetyl- β -D-galactosaminyltransferase from guinea-pig kidney involved in the biosynthesis of Sd^a antigen associated with Tamm-Horsfall glycoprotein. *Carbohydr Res* 151:65-76, 1986
46. Cavallone D, Malagolini N, Minni F, Serafini-Cessi F: Distribution in human kidney of Tamm-Horsfall glycoprotein and of glycosyltransferases involved in the biosynthesis of the Sd^a antigen [in Italian]. *G Ital Nefrol* 4:418-424, 2001
47. Dall'Olio F, de Kañter FJJ, van den Eijnden DH, Serafini-Cessi F: Structural analysis of the preponderant high-mannose oligosaccharides of human Tamm-Horsfall glycoprotein. *Carbohydr Res* 178:327-332, 1988
48. Serafini-Cessi F, Malagolini N, Hoops TC, Rindler MJ: Biosynthesis and oligosaccharide processing of human Tamm-Horsfall glycoprotein permanently expressed in HeLa cells. *Biochem Biophys Res Commun* 194:784-790, 1993
49. Smagula RM, van Halbeek H, Decker JM, Muchmore AV, Moody CE, Sherblom AP: Pregnancy-associated changes in oligomannose oligosaccharides of human and bovine uromodulin (Tamm-Horsfall glycoprotein). *Glycoconjugate J* 7:609-624, 1990
50. Firon N, Ofek I, Sharon N: Carbohydrate-binding sites of the mannose-specific fimbrial lectins of enterobacteria. *Infect Immun* 43:1088-1090, 1984
51. Easton RL, Patankar MS, Clark GF, Morris HR, Dell A: Pregnancy-associated changes in the glycosylation of Tamm-Horsfall glycoprotein; Expression of sialyl Lewis^x sequences on core 2 type *O*-glycans derived from uromodulin. *J Biol Chem* 275:21928-21938, 2000
52. Muchmore AV, Shifrin S, Decker JM: In vitro evidence that carbohydrate moieties derived from uromodulin, an 85,000 dalton immunosuppressive glycoprotein isolated from human pregnancy urine, are immunosuppressive in the absence of intact protein. *J Immunol* 138:2547-2553, 1987
53. Olczak T, Kubicz A, Kokot F, Dulawa J: Tamm-Horsfall protein isolated from urine of pregnant and non-

pregnant women has similar oligosaccharides. *Eur J Clin Invest* 28:475-482, 1998

54. Olczak M, Olczak T: Oligosaccharides released by hydrazinolysis from Tamm-Horsfall protein of various human donors contain similar high-mannose glycans. *Clin Chim Acta* 282:35-44, 1999

55. van Rooijen JJM, Hermentin P, Kamerling JP, Vliegenthart JFG: The patterns of the complex- and oligomannose-type glycans of uromodulin (Tamm-Horsfall glycoprotein) in the course of pregnancy. *Glycoconjugate J* 18:539-546, 2001

56. Schenk EA, Schwartz RH, Lewis RA: Tamm-Horsfall mucoprotein: I. Localization in the kidney. *Lab Invest* 25:92-95, 1971

57. Hoyer JR, Sisson SP, Vernier RL: Tamm-Horsfall glycoprotein: Ultrastructural immunoperoxidase localization in rat kidney. *Lab Invest* 41:168-173, 1979

58. Seiler MW, Hoyer JR: Ultrastructural studies of tubulointerstitial immune complex nephritis in rats immunized with Tamm-Horsfall protein. *Lab Invest* 45:321-327, 1981

59. Sikri KL, Foster CL, Bloomfield FJ, Marshall RD: Localization by immunofluorescence and by light- and electron-microscopic immunoperoxidase techniques of Tamm-Horsfall glycoprotein in adult hamster kidney. *Biochem J* 181:525-532, 1979

60. Sikri KL, Foster CL, MacHugh N, Marshall RD: Localization of Tamm-Horsfall glycoprotein in the human kidney using immunofluorescence and immunoelectron-microscopical techniques. *J Anat* 132:597-605, 1981

61. Bachmann S, Koeppen-Hagemann I, Kriz W: Ultrastructural localization of Tamm-Horsfall glycoprotein (THP) in rat kidney as revealed by protein-gold immunocytochemistry. *Histochemistry* 83:531-538, 1985

62. Bachmann S, Metzger R, Bunnemann B: Tamm-Horsfall protein-mRNA synthesis is localized to the thick ascending limb of Henle's loop in rat kidney. *Histochemistry* 94:517-523, 1990

63. Gokhale JA, Glenton PA, Khan SR: Characterization of Tamm-Horsfall protein in a rat nephrolithiasis model. *J Urol* 166:1492-1497, 2001

64. Brown DA, Rose JK: Sorting of GPI-anchored protein to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* 68:533-544, 1992

65. Benting JH, Rietveld AG, Simons K: N-Glycans mediate the apical sorting of a GPI-anchored, raft associated protein in Madin-Darby canine kidney cells. *J Cell Biol* 146:313-320, 1999

66. Horton JK, Davies M, Woodhead JS, Weeks I: A new and rapid immunochemiluminometric assay for the measurement of Tamm-Horsfall glycoprotein. *Clin Chim Acta* 174:225-237, 1988

67. Kreft B, Jabs WJ, Laskay T, et al: Polarized expression of Tamm-Horsfall protein by renal tubular epithelial cells activates human granulocytes. *Infect Immun* 70:2650-2656, 2002

68. Hoyer JR, Resnick JS, Michael AF, Vernier RL: Ontogeny of Tamm-Horsfall urinary glycoprotein. *Lab Invest* 30:757-761, 1974

69. Phimister GM, Marshall RD: Tamm-Horsfall glycoprotein in human amniotic fluid. *Clin Chim Acta* 128:261-269, 1983

70. Ecelbarger CA, Terris J, Hoyer JR, Nielsen S, Wade JB, Knepper MA: Localization and regulation of the renal Na(+)-K(+)-2Cl- cotransporter, BSC-1. *Am J Physiol* 271:F619-F628, 1996

71. Kobayashi K, Fukuoka S: Conditions for solubilization of Tamm-Horsfall protein/uromodulin in human urine and establishment of a sensitive and accurate enzyme-linked immunosorbent assay (ELISA) method. *Arch Biochem Biophys* 388:113-120, 2001

72. Dawnay AB, Thornley C, Cattell WR: An improved radioimmunoassay for urinary Tamm-Horsfall glycoprotein. Investigation and resolution of factors affecting its quantification. *Biochem J* 206:461-465, 1982

73. Thornley C, Dawnay A, Cattell WR: Human Tamm-Horsfall glycoprotein: Urinary and plasma levels in normal subjects and patients with renal disease determined by a fully validated radioimmunoassay. *Clin Sci (Lond)* 68:529-535, 1985

74. Lynn KL, Shenkin A, Marshall RD: Factors affecting excretion of human urinary Tamm-Horsfall glycoprotein. *Clin Sci (Lond)* 62:21-26, 1982

75. Ying WZ, Sanders PW: Dietary salt regulates expression of Tamm-Horsfall glycoprotein in rats. *Kidney Int* 54:1150-1156, 1998

76. Stevenson FK, Cleave AJ, Kent PW: The effect of ions on the viscometric and ultracentrifugal behaviour of Tamm-Horsfall glycoprotein. *Biochim Biophys Acta* 236:59-66, 1971

77. Wiggins RC: Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under different physicochemical conditions. *Clin Chim Acta* 162:329-340, 1987

78. Serafini-Cessi F, Bellabarba G, Malagolini N, Dall'Olio F: Rapid isolation of Tamm-Horsfall glycoprotein (uromodulin) from human urine. *J Immunol Methods* 120:185-189, 1989

79. Cavallone D, Malagolini N, Frasca GM, Stefoni S, Serafini-Cessi F: Salt-precipitation method does not isolate to homogeneity Tamm-Horsfall glycoprotein from urine of proteinuric patients and pregnant women. *Clin Biochem* 35:405-410, 2002

80. Rhodes DCJ, Hinsman EJ, Rodhes JA: Tamm-Horsfall glycoprotein binds IgG with high affinity. *Kidney Int* 44:1014-1021, 1993

81. Rhodes DC, Hinsman EJ, Rodhes JA: Errors in reported association between Tamm-Horsfall protein and IgG. *Kidney Int* 56:759, 1999 (letter)

82. Olczak T, Olczak M, Dereniowska M, Strzelczyk R, Kubicz A: Alterations of the sugar moiety of Tamm-Horsfall protein in children with malignancies of lymphoid cells. *Electrophoresis* 20:1382-1389, 1999

83. Olczak T, Olczak M, Kubicz A, Dulawa J, Kokot F: Composition of the sugar moiety of Tamm-Horsfall protein in patients with urinary diseases. *Int J Clin Lab Res* 29:68-74, 1999

84. Sanders PW, Booker BB: Pathobiology of cast nephropathy from human Bence Jones proteins. *J Clin Invest* 89:630-639, 1992

85. Rhodes DC: Binding of Tamm-Horsfall protein to complement 1q measured by ELISA and resonant mirror

biosensor techniques under various ionic-strength conditions. *Immunol Cell Biol* 78:474-482, 2000

86. Rhodes DC: Binding of Tamm-Horsfall protein to complement 1q and complement 1, including influence of hydrogen-ion concentration. *Immunol Cell Biol* 80:558-566, 2002

87. Serafini-Cessi F, Dall'Olio F: Affinity of leucoagglutinin (L-PHA), compared with *Dolichos biflorus* lectin and Con A, for human Tamm-Horsfall glycopeptide, in Bog-Hansen TH (ed): *Lectins—Biology, Biochemistry, Clinical Biochemistry*, vol II. Berlin, Germany, de Gruyter, 1982, pp 367-379

88. Abbondanza A, Franceschi C, Licastro F, Serafini-Cessi F: Properties of glycopeptide isolated from human Tamm-Horsfall glycoprotein. *Biochem J* 187:525-528, 1980

89. Cummings RD, Kornfeld S: Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized *Phaseolus vulgaris* leucoagglutinating and erythroagglutinating lectins. *J Biol Chem* 257:11230-11234, 1982

90. Hammarström S, Hammarström ML, Sundblad G, Arnarp J, Lönngren J: Mitogenic leucoagglutinin from *Phaseolus vulgaris* binds to a pentasaccharide unit in *N*-acetyllactosamine-type glycoprotein glycans. *Proc Natl Acad Sci U S A* 79:1611-1615, 1982

91. Sherblom AP, Decker JM, Muchmore AV: The lectin-like interaction between recombinant tumor necrosis factor and uromodulin. *J Biol Chem* 263:5418-5424, 1988

92. Moonen P, Williamson K: Bioassay for interleukin-1 inhibitors. *J Immunol Methods* 102:283-284, 1987

93. Dall'Olio F, Chiricolo M, Malagolini N, Franceschi C, Serafini-Cessi F: Immunosuppressive activity of Tamm-Horsfall glycoprotein oligosaccharides: Effect of removal of outer sugars and conjugation with a protein carrier. *Cell Immunol* 137:303-315, 1991

94. Tandai-Hiruma M, Endo T, Kobata A: Detection of novel carbohydrate binding activity of interleukin-1. *J Biol Chem* 274:4459-4466, 1999

95. Horton JK, Davies M, Topley N, Thomas D, Williams JD: Activation of the inflammatory response of neutrophils by Tamm-Horsfall glycoprotein. *Kidney Int* 37:717-726, 1990

96. Thomas DBL, Davies M, Peters JR, Williams JD: Tamm-Horsfall protein binds to a single class of carbohydrate specific receptors on human neutrophils. *Kidney Int* 44:423-429, 1993

97. Toma G, Bates JM Jr, Kumar S: Uromodulin (Tamm-Horsfall protein) is a leukocyte adhesion molecule. *Biochem Biophys Res Commun* 200:275-282, 1994

98. Yu CL, Lin WM, Liao TS, Tsai CY, Sun KH, Chen KH: Tamm-Horsfall glycoprotein (THG) purified from normal human pregnancy urine increases phagocytosis, complement receptor expressions and arachidonic acid metabolism of polymorphonuclear neutrophils. *Immunopharmacology* 24:181-190, 1992

99. Yu CL, Tsai CY, Lin WM, Liao TS, Chen HL, Sun KH: Tamm-Horsfall urinary glycoprotein enhances monokine release and augments lymphocyte proliferation. *Immunopharmacology* 26:249-258, 1993

100. Su S-J, Chang K-L, Lin T-M, Huang Y-H, Yeh T-M: Uromodulin and Tamm-Horsfall protein induce human mono-

cytes to secrete TNF and express tissue factor. *J Immunol* 158:3449-3456, 1997

101. Thomas DBL, Davies M, Williams JD: Release of gelatinase and superoxide from human mononuclear phagocytes in response to particulate Tamm-Horsfall protein. *Am J Pathol* 142:249-260, 1993

102. Fontan E, Briend E, Saklani-Jusforgues H, D'Alayer J, Vandekerckhove J, Fauve RM: A 92-kDa human immunostimulatory protein. *Proc Natl Acad Sci U S A* 91:8353-8357, 1994

103. Fontan E, Jusforgues-Saklani H, Briend E, Fauve RM: Purification of a 92kDa human immunostimulating glycoprotein obtained from the Tamm-Horsfall glycoprotein. *J Immunol Methods* 187:81-84, 1995

104. Orskov I, Ferencz A, Orskov F: Tamm-Horsfall protein or uromucoid is the normal urinary slime that traps type 1 fimbriated *Escherichia coli*. *Lancet* 1:887, 1980 (letter)

105. Parkkinen J, Virkola R, Korhonen TK: Identification of factors in human urine that inhibit the binding of *Escherichia coli* adhesins. *Infect Immun* 56:2623-2630, 1988

106. Reinhart HH, Obedeau N, Sobel JD: Quantitation of Tamm-Horsfall protein binding to uropathogenic *Escherichia coli* and lectins. *J Infect Dis* 162:1335-1340, 1990

107. Pak J, Pu Y, Zhang ZT, Hasty DL, Wu XR: Tamm-Horsfall protein binds to type 1 fimbriated *Escherichia coli* and prevents *E. coli* from binding to uroplakin Ia and Ib receptors. *J Biol Chem* 276:9924-9930, 2001

108. Barnett BJ, Stephens DS: Urinary tract infection: An overview. *Am J Med Sci* 314:245-249, 1997

109. Sharon N: Bacterial lectins, cell-cell recognition and infectious disease. *FEBS Lett* 217:145-157, 1987

110. Martinez JJ, Mulvey MA, Schilling JD, Pinkner JS, Hultgren SJ: Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *EMBO J* 19:2803-2812, 2000

111. Wu XR, Lin JH, Walz T, et al: Mammalian uroplakins: A group of highly conserved urothelial differentiation-related membrane proteins. *J Biol Chem* 269:13716-13724, 1994

112. Wu XR, Medina JJ, Sun TT: Selective interactions of UP Ia and UP Ib, two members of the transmembrane 4 superfamily, with distinct single transmembrane-domained proteins in differentiated urothelial cells. *J Biol Chem* 270:29752-29759, 1995

113. Wu XR, Sun TT, Medina JJ: In vitro binding of type 1-fimbriated *Escherichia coli* to uroplakins Ia and Ib: Relation to urinary tract infections. *Proc Natl Acad Sci U S A* 93:9630-9635, 1996

114. Mulvey MA, Lopez-Boado YS, Wilson CL, et al: Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* 282:1494-1497, 1998

115. Hart TC, Gorry MC, Hart PS, et al: Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. *J Med Genet* 39:882-892, 2002

116. Wenk RE, Bhagavan BS, Rudert J: Tamm-Horsfall uromucoprotein and the pathogenesis of casts, reflux nephropathy, and nephritides. *Pathobiol Annu* 11:229-257, 1981

117. McQueen EG: The nature of urinary casts. *J Clin Pathol* 15:367-373, 1962

118. McQueen EG: Composition of urinary casts. *Lancet* 1:397-398, 1966
119. Fletcher AP, McLaughlin JE, Ratcliffe WA, Woods DA: The chemical composition and electron microscopic appearance of a protein derived from urinary casts. *Biochim Biophys Acta* 214:299-308, 1970
120. Fairley JK, Owen JE, Birch DF: Protein composition of urinary casts from healthy subjects and patients with glomerulonephritis. *Br Med J* 287:1838-1840, 1983
121. Fogazzi GB, Testanera G: The farsighted studies of the Italian Carlo L. Roida (1844-1877) on the nature of urinary casts. *Am J Nephrol* 22:300-308, 2002
122. Huang ZQ, Kirk KA, Connelly KG, Sanders PW: Bence Jones proteins bind to a common peptide segment of Tamm-Horsfall glycoprotein to promote heterotypic aggregation. *J Clin Invest* 92:2975-2983, 1993
123. Wangsiripaisan A, Gengaro PE, Edelstein CL, Schrier RW: Role of polymeric Tamm-Horsfall protein in cast formation: Oligosaccharide and tubular fluid ions. *Kidney Int* 59:932-940, 2001
124. Sanders PW, Booker BB, Bishop JB, Cheung HC: Mechanism of intranephronal proteinaceous cast formation by low molecular weight proteins. *J Clin Invest* 85:570-576, 1990
125. Huang ZQ, Sanders PW: Biochemical interaction between Tamm-Horsfall glycoprotein and Ig light chains in the pathogenesis of cast nephropathy. *Lab Invest* 73:810-817, 1995
126. Huang ZQ, Sanders PW: Localization of a single binding site for immunoglobulin light chains on human Tamm-Horsfall glycoprotein. *J Clin Invest* 99:732-736, 1997
127. Ying WZ, Sanders PW: Mapping the binding domain of immunoglobulin light chains for Tamm-Horsfall protein. *Am J Pathol* 158:1859-1866, 2001
128. Lebouilleux M, Lelongt B, Mougenot B, et al: Protease resistance and binding of Ig light chains in myeloma-associated tubulopathies. *Kidney Int* 48:72-79, 1995
129. Freedman SD, Sakamoto K, Venu RP: GP2, the homologue to the renal cast protein uromodulin, is a major component of intraductal plugs in chronic pancreatitis. *J Clin Invest* 92:83-90, 1993
130. Coe FL, Parks JH, Asplin JR: The pathogenesis and treatment of kidney stones. *N Engl J Med* 327:1141-1152, 1992
131. Kok DJ, Khan SR: Calcium oxalate nephrolithiasis, a free or fixed particle disease. *Kidney Int* 46:847-854, 1994
132. Lieske JC, Norris R, Swift H, Toback FG: Adhesion, internalization and metabolism of calcium oxalate monohydrate crystals by renal epithelial cells. *Kidney Int* 52:1291-1301, 1997
133. Marangella M, Vitale C, Petrarulo M, Bagnis C, Bruno M, Ramello A: Renal stones: From metabolic to physicochemical abnormalities. How useful are inhibitors? *J Nephrol* 13:S51-S60, 2000 (suppl 3)
134. Keutel HJ: Localization of uromucoid in human kidney and in sections of human kidney stone with the fluorescent antibody technique. *J Histochem Cytochem* 13:155-160, 1965
135. Grant AM, Baker LR, Neuberger A: Urinary Tamm-Horsfall glycoprotein in certain kidney diseases and its content in renal and bladder calculi. *Clin Sci* 44:377-384, 1973
136. Hess B, Kok DJ: Nucleation, growth and aggregation of stone forming crystals, in Coe FL, Favus MJ, Fak CYC, Parks JH, Preminger GM (eds): *Kidney Stones: Medical and Surgical Management*. Philadelphia, PA, Lippincott-Raven, 1996, pp 3-32
137. Miyake O, Yoshioka T, Yoshimura K, et al: Expression of Tamm-Horsfall protein in stone-forming rat models. *Br J Urol* 81:14-19, 1998
138. Worcester EM: Urinary calcium oxalate crystal growth inhibitors. *J Am Soc Nephrol* 5:S46-S53, 1994
139. Hess B, Nakagawa Y, Coe FL: Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. *Am J Physiol* 257 (Ren Fluid Electrolyte Physiol) 26:99-106, 1989
140. Grover PK, Moritz RL, Simpson RJ, Ryall RL: Inhibition of growth and aggregation of calcium oxalate crystals in vitro: A comparison of four human proteins. *Eur J Biochem* 253:637-644, 1998
141. Beshensky AM, Wesson JA, Worcester EM, Sorokina EJ, Snyder CJ, Kleinman JG: Effects of urinary macromolecules on hydroxyapatite crystal formation. *J Am Soc Nephrol* 12:2108-2116, 2001
142. Fellstrom B, Danielson BG, Ljunghall S, Wikstrom B: Crystal inhibition: The effects of polyanions on calcium oxalate crystal growth. *Clin Chim Acta* 158:229-235, 1986
143. Michelacci YM, Boim MA, Bergamaschi CT, Rovigatti RM, Schor N: Possible role for chondroitin sulfate in urolithiasis: In vivo studies in an experimental model. *Clin Chim Acta* 208:1-8, 1992
144. Worcester EM, Blumenthal SS, Beshensky AM, Lewand DL: The calcium oxalate crystal growth inhibitor protein produced by mouse kidney cortical cells in culture is osteopontin. *J Bone Miner Res* 7:1029-1036, 1992
145. Shiraga H, Min W, VanDusen WJ, et al: Inhibition of calcium oxalate crystal growth in vitro by uropontin: Another member of the aspartic acid-rich protein superfamily. *Proc Natl Acad Sci U S A* 89:426-430, 1992
146. Ryall RL, Grover PK, Stapleton AM, Barrell DK, Tang Y, Simpson RJ: The urinary F1^{act} activation peptide of human prothrombin is a potent inhibitor of calcium oxalate crystallization in undiluted human urine in vitro. *Clin Sci* 89:533-541, 1995
147. Lieske JC, Toback FG, Deganello S: Sialic acid-containing glycoproteins on renal cells determine nucleation of calcium oxalate dihydrate crystals. *Kidney Int* 60:1784-1791, 2001
148. Koka RM, Huang E, Lieske JC: Adhesion of uric acid crystals to the surface of renal epithelial cells. *Am J Physiol Ren Physiol* 278:F989-F998, 2000
149. Marengo SR, Chen DH, Kaung HL, Resnick MI, Yang L: Decreased renal expression of the putative calcium oxalate inhibitor Tamm-Horsfall protein in the ethylene glycol rat model of calcium oxalate urolithiasis. *J Urol* 167:2192-2197, 2002
150. Knorle R, Schnierle P, Koch A, et al: Tamm-Horsfall glycoprotein: Role in inhibition and promotion of renal calcium oxalate stone formation studied with Fourier-transform infrared spectroscopy. *Clin Chem* 40:1739-1743, 1994

151. Schnierle P: A simple diagnostic method for the differentiation of Tamm-Horsfall glycoproteins from healthy probands and those from recurrent calcium oxalate renal stone formers. *Experientia* 51:1068-1072, 1995
152. Boeve ER, Cao LC, De Bruijn WC, Robertson WG, Romijn JC, Schroder FH: Zeta potential distribution on calcium oxalate crystal and Tamm-Horsfall protein surface analyzed with Doppler electrophoretic light scattering. *J Urol* 152:531-536, 1994
153. Grover PK, Resnick MI: Evidence for the presence of abnormal proteins in the urine of recurrent stone formers. *J Urol* 153:1716-1721, 1995
154. Trewick AL, Rumsby G: Isoelectric focusing of native urinary uromodulin (Tamm-Horsfall protein) shows no physicochemical differences between stone formers and non-stone formers. *Urol Res* 27:250-254, 1999
155. Hallson PC, Choong SK, Kasidas GP, Samuelli CT: Effects of Tamm-Horsfall protein with normal and reduced sialic acid content upon the crystallization of calcium phosphate and calcium oxalate in human urine. *Br J Urol* 80:533-538, 1997
156. Chen WC, Lin HS, Chen HY, Shih CH, Li CW: Effects of Tamm-Horsfall protein and albumin on calcium oxalate crystallization and importance of sialic acids. *Mol Urol* 5:1-5, 2001
157. Maslamani S, Glenton PA, Khan SR: Changes in urine macromolecular composition during processing. *J Urol* 164:230-236, 2000
158. Heptinstall RH: Interstitial nephritis. A brief review. *Am J Pathol* 83:213-236, 1976
159. Zager RA, Cotran RS, Hoyer JR: Pathologic localization of Tamm-Horsfall protein in interstitial deposits in renal disease. *Lab Invest* 38:52-57, 1978
160. Resnick JS, Sisson S, Vernier RL: Tamm-Horsfall protein: Abnormal localization in renal disease. *Lab Invest* 38:550-555, 1978
161. Cotran RS, Galvanek E: Immunopathology of human tubulo-interstitial diseases: Localization of immunoglobulins complement and Tamm-Horsfall protein. *Contrib Nephrol* 16:126-131, 1979
162. Cohen AH, Border WA, Rayfer J, Dumke A, Glascock RJ: Interstitial Tamm-Horsfall protein in rejecting renal allografts: Identification and morphologic pattern of injury. *Lab Invest* 50:519-525, 1984
163. Wilson CB: Nephritogenic tubulointerstitial antigens. *Kidney Int* 39:501-517, 1991
164. Hoyer JR: Tubulointerstitial immune complex nephritis in rats immunized with Tamm-Horsfall protein. *Kidney Int* 17:284-292, 1980
165. Mayrer AR, Kashgarian M, Ruddle NH, et al: Tubulointerstitial nephritis and immunologic responses to Tamm-Horsfall protein in rabbits challenged with homologous urine or Tamm-Horsfall protein. *J Immunol* 128:2634-2642, 1982
166. Fasth A, Hoyer JR, Seiler MW: Renal tubular immune complex formation in mice immunized with Tamm-Horsfall protein. *Am J Pathol* 125:555-562, 1986
167. Nagai T, Nagai T: Tubulointerstitial nephritis by Tamm-Horsfall glycoprotein or egg white component. *Nephron* 47:134-140, 1987
168. Venkatachalam MA, Karnovsky MJ: Extravascular protein in the kidney: An ultrastructural study of its relation to renal peritubular capillary permeability using protein tracers. *Lab Invest* 27:435-444, 1972
169. Chambers R, Groufsky A, Hunt JS, Lynn KL, McGiven AR: Relationship of abnormal Tamm-Horsfall glycoprotein localization to renal morphology and function. *Clin Nephrol* 26:21-26, 1986
170. Howie AJ, Brewer DB: Extra-tubular deposits of Tamm-Horsfall protein in renal allografts. *J Pathol* 139:193-206, 1983
171. Howie AJ: Tamm-Horsfall protein outside the kidney. *J Pathol* 153:399-404, 1987
172. Carr RA, Newman J, Antonakopoulos GN, Parkinson MC: Lesions produced by the extravasation of urine from the upper urinary tract. *Histopathology* 30:335-340, 1997
173. Dziukas LJ, Sterzel RB, Hodson CJ, Hoyer JR: Renal localization of Tamm-Horsfall protein in unilateral obstructive uropathy in rats. *Lab Invest* 47:185-193, 1982
174. Fasth AL, Hoyer JR, Seiler MW: Extratubular Tamm-Horsfall protein deposits induced by ureteral obstruction in mice. *Clin Immunol Immunopathol* 47:47-61, 1988
175. Cavallone D, Malagolini N, Serafini-Cessi F: Binding of human neutrophils to cell-surface anchored Tamm-Horsfall glycoprotein in tubulointerstitial nephritis. *Kidney Int* 55:1787-1799, 1999
176. Morita Y, Ikeguchi H, Nakamura J, Hotta N, Yuzawa Y, Matsuo S: Complement activation products in the urine from proteinuric patients. *J Am Soc Nephrol* 11:700-707, 2000
177. Lynn KL, Marshall RD: Excretion of Tamm-Horsfall glycoprotein in renal disease. *Clin Nephrol* 22:253-257, 1984
178. Gläuser A, Hochreiter W, Jaeger P, Hess B: Determinants of urinary excretion of Tamm-Horsfall protein in non-selected kidney stone formers and healthy subjects. *Nephrol Dial Transplant* 15:1580-1587, 2000
179. Tsai CY, Wu TH, Yu CL, Lu JY, Tsai YY: Increased excretions of beta₂-microglobulin, IL-6, and IL-8 and decreased excretion of Tamm-Horsfall glycoprotein in urine of patients with active lupus nephritis. *Nephron* 85:207-214, 2000
180. Schroter J, Timmermans G, Seyberth HW, Greven J, Bachmann S: Marked reduction of Tamm-Horsfall protein synthesis in hyperprostaglandin E-syndrome. *Kidney Int* 44:401-410, 1993
181. Romero MC, Nocera S, Nesse AB: Decreased Tamm-Horsfall protein in lithiasic patients. *Clin Biochem* 30:63-67, 1997
182. Ganter K, Bongartz D, Hesse A: Tamm-Horsfall protein excretion and its relation to citrate in urine of stone-forming patients. *Urology* 53:492-495, 1999
183. Bichler KH, Mittermüller B, Strohmaier WL, Feil G, Eipper E: Excretion of Tamm-Horsfall protein in patients with uric acid stones. *Urol Int* 62:87-92, 1999
184. Torffvit O, Kamper AL, Strandgaard S: Tamm-Horsfall protein in urine after uninephrectomy/transplantation in kidney donors and their recipients. *Scand J Urol Nephrol* 31:555-559, 1997
185. Bernard AM, Ouled AA, Lauwerys RR, Lambert A, Vandeleeuw B: Pronounced decrease of Tamm-Horsfall proteinuria in diabetics. *Clin Chem* 33:1264-1267, 1987

186. Lauwerys R, Bernard A: Preclinical detection of nephrotoxicity: Description of the tests and appraisal of their health significance. *Toxicol Lett* 46:13-29, 1989
187. Torffvit O, Agardh CD: Tubular secretion of Tamm-Horsfall protein is decreased in type 1 (insulin-dependent) diabetic patients with diabetic nephropathy. *Nephron* 65:227-231, 1993
188. Torffvit O, Agardh CD: Urinary excretion rate of NCI and Tamm-Horsfall protein in the microalbuminuric type I diabetic patient. *J Diabetes Complications* 8:77-83, 1994
189. Below AA, Chakraborty J, Khuder SH, Haselhuhn GD: Evaluation of urinary Tamm-Horsfall protein in post-menopausal diabetic women. *J Diabetes Complications* 13:204-210, 1999
190. Holmquist P, Torffvit O, Jorgensen PE, Torring N, Nexø E, Sjoblad S: Early urinary changes in Tamm-Horsfall protein and epidermal growth factor in diabetic children. *Pediatr Nephrol* 16:488-492, 2001
191. Zimmerhackl LB, Pfeiderer S, Kinne R, Manz F, Schuler G, Brandis M: Tamm-Horsfall-protein excretion as a marker of ascending limb transport indicates early renal tubular damage in diabetes mellitus type I. *J Diabetes Complications* 5:112-114, 1991
192. Pfeiderer S, Zimmerhackl LB, Kinne R, Manz F, Schuler G, Brandis M: Renal proximal and distal tubular function is attenuated in diabetes mellitus type 1 as determined by the renal excretion of alpha 1-microglobulin and Tamm-Horsfall protein. *Clin Invest* 71:972-977, 1993
193. Catalano C, Torffvit O: Urinary excretion of Tamm-Horsfall protein in normotensive, normo-albuminuric type 1 diabetic patients. *Nephron* 72:436-441, 1996
194. Thulesen J, Jorgensen PE, Torffvit O, Nexø E, Poulsen SS: Urinary excretion of epidermal growth factor and Tamm-Horsfall protein in three rat models with increased renal excretion of urine. *Regul Pept* 72:179-186, 1997
195. Agardh CD, Stenram U, Torffvit O, Agardh E: Effects of inhibition of glycation and oxidative stress on the development of diabetic nephropathy in rats. *J Diabetes Complications* 16:395-400, 2002
196. Dahan K, Fuchshuber A, Adamis S, et al: Familial juvenile hyperuricemic nephropathy and autosomal dominant medullary cystic kidney disease type 2: Two facets of the same disease? *J Am Soc Nephrol* 12:2348-2357, 2001
197. Scolari F, Puzzer D, Amoroso A, et al: Identification of a new locus for medullary cystic disease, on chromosome 16p12. *Am J Hum Genet* 64:1655-1660, 1999
198. Stiburkova B, Majewski J, Sebesta I, Zhang W, Ott J, Kmoch S: Familial juvenile hyperuricemic nephropathy: Localization of the gene on chromosome 16p11.2 and evidence for genetic heterogeneity. *Am J Hum Genet* 66:1989-1994, 2000
199. Zbikowska HM, Soukhareva N, Behnam R, Lubon H, Hammond D, Soukharev S: Uromodulin promoter directs high-level expression of biologically active human alpha₁-antitrypsin into mouse urine. *Biochem J* 365:7-11, 2002
200. Zbikowska HM, Soukhareva N, Behnam R, et al: The use of the uromodulin promoter to target production of recombinant proteins into urine of transgenic animals. *Transgenic Res* 11:425-435, 2002
201. Kerr DE, Liang F, Bondioli KR, et al: The bladder as a bioreactor: Urothelium production and secretion of growth hormone into urine. *Nat Biotechnol* 16:75-79, 1998
202. Rossant J, Nagy A: Genome engineering: The new mouse genetics. *Nat Med* 1:592-594, 1995
203. Stricklett PK, Taylor D, Nelson RD, Kohan DE: Thick ascending limb-specific expression of Cre recombinase. *Am J Physiol Ren Physiol* 285:F33-F39, 2003
204. Kumar S, Muchmore A: Tamm-Horsfall protein-uromodulin (1950-1999). *Kidney-Int* 37:1395-1401, 1990
205. Kornfeld R, Kornfeld S: Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem* 84:631-664, 1985
206. Mulvey MA: Adhesion and entry of uropathogenic *Escherichia coli*. *Cell Microbiol* 4:257-271, 2002